Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Foreword

Since there are well over 1000 immunologists in the Federal Republic of Germany, it is impossible to provide a complete picture of immunology and this issue can highlight only some areas of research. As a guide for selecting the topics for individual articles we relied heavily on the published abstracts of the most recent proceedings of the German Society of Immunology, since these popular annual meetings are well attended and the collections of abstracts reflect major areas of immunological research in West Germany. We gratefully acknowledge the time and efforts of all the authors who wrote these summaries. It is clear that the names, addresses and research work of so many immunologists cannot be presented in a single issue and we are sorry for this limitation. The Society of Immunology maintains a listing of its members and the Administrative Office (H. Kirchner, Department of Immunology, University of Lubeck) can provide current addresses of its members. It should also be mentioned that the society has many active members in other countries, including Austria and Switzerland, whose work is not included in this issue. Despite these shortcomings we hope that readers will acquire a feeling for happenings in immunology today in the Federal Republic of Germany.

Immunology Today is indebted to Dolores J. Schendel and Matthias Cramer for their help in compiling this supplement.

Introduction to the 7th International Congress of Immunology

Klaus Eichmann

The 7th International Congress of Immunology will be held in the city of Berlin (West) on 30 July – 5 August, 1989 and is being organized by the Gesellschaft für Immunologie. Based on estimates from the number of submitted abstracts, between 7000 and 8000 immunologists are expected to attend. This amounts to approximately one third of the total membership of the 39 national societies that constitute the International Union of Immunological Societies (IUIS). The burden of organizing such a conference is more than fully compensated for by the overwhelming worldwide interest.

The congress is being held under the auspices of the IUIS which was founded in 1969 by ten national immunological societies including the Gesellschaft für Immunologie. The IUIS has grown rapidly to its present size and strength. Without the IUIS and the scientists who devote much of their time and effort to this organization, it would not be possible to have an international congress such as this.

Germany and the city of Berlin have a great past in immunology. One hundred years ago, antibodies were first discovered here by Emil von Behring and his Japanese colleague S. Kitasato. Names such as Robert Koch, Rodolf Virchow and Paul Ehrlich need no special explanations. Today, immunologists in Germany are proud to have been entrusted, by the international community of immunologists, with the organization of its principal and most comprehensive meeting. During the conference, we shall honor the great past of immunology in Germany with a historical exhibition. For the scientific program, the unanimous opinion of the Organizing Committee was to avoid any sort of national representation but to ask the very best in the international community to serve as chairpersons and speakers. Immunology as it stands in present day Germany is reviewed in this issue of *Immunology Today*.

No other scientific discipline has contributed quite as much in recent years as immunology has to both basic biology and its application in medicine. Our knowledge of the biology of genes and their products, of cells and multicellular organisms and also our diagnostic and therapeutic skills in practical medicine have been greatly furthered by information drawn from immunological research. Immunology has also become more heterogeneous than perhaps any other scientific discipline. No contemporary immunologist can claim to have a comprehensive understanding of his entire field. Faced with this situation – which has both merits and difficulties – the International Congress of Immunology provides a singular opportunity for the individual scientist to recognize the major avenues of immunological research and thus to define individual pathways. More than ever before do we need this forum of mutual information and learning.

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The development of the scientific program of the 7th International Congress of Immunology

F. Melchers

The immune system must certainly be one of the most intensively studied parts of the body. It is estimated that over 100 000 scientists all over the world work in the many different areas of immunology. Basic questions about the structure and function of the immune system at the molecular, cellular and physiological levels are asked in many different species. Clinical observations of immunodeficiencies and autoimmune diseases, in inflammations and allergies, help in the search for an explanation, and eventually for a therapy, within a rapidly expanding understanding of the immune system. This increased knowledge encourages new strategies in transplantation and in the fight against bacterial, parasitic and viral infections as well as against cancer. New industries have developed which apply this knowledge in diagnosis and therapy.

From the first discussions in February, 1986 (see Box 1), the program committee (see Final Program of the Congress) decided that its main task was to assemble under one roof, in one program, these many different and often diverging interests in the immune system. In an attempt to bring together many scientists and clinicians with different training, introductory lectures at the beginning of each symposium are given by a chairperson who has been asked to summarize the current status of the field to be covered by the symposium. This should offer an immunologist not specializing in the given field an opportunity to update his/her knowledge of the recent developments in this area. The work of many colleagues, and to a limited extent that of the chairperson introducing the symposium, should be covered. No other activities of the congress, such as workshops, are held concurrently with these introductions. Since the program includes 27 symposia, as well as 130 workshops, this will allow every tireless participant to hear 11 of the 27 introductions – a reasonable compromise considering the frustrating reality that it would take 80 days to participate in *all* events of the congress.

The program committee organized its search for topics, speakers and chairpersons by forming 13 subcommittees covering various aspects of basic and clinical immunology. Their suggestions were sent to some 200 international colleagues (see Final Program for International Advisers) who made additional suggestions – with the aim of incorporating the most exciting, latest developments and talent into the program.

Box 1. The formation of the scientific program of the 7th International Congress of Immunology

Fall 1985	Chairman and Advisory Board of Society for Immunology select F. Melchers as chairman of program committee.
February 1986	Marburg Chairman and Advisory Board discuss with program chairman the format and scope of the program, selection of program committee members, formation of 13 subcommittees with 17 chairpersons and nearly 60 members in total.
November 1986 – February 1987	Subcommittees discuss the program of various subspecialities of immunology and propose 34 symposia and 154 workshops.
September 1987	Ulm The total program suggested by the 13 subcommittees is discussed with all national advisers; the program is reduced to 23 symposia and 90 workshops. Approximately 200 international advisers to the congress are selected.
Winter 1987–1988	Suggestions from international advisers are incorporated into the scientific program.
April 1988	Elmau Symposium on The Immune System in 1988 is held. Twenty-two summary talks are given by the international advisers on different areas of immunology. Program is discussed with a selected group of international and all national advisers. Final selection of topics occurs.
May 1988	Basel Topics and speakers are finally selected by all subcommittee chairpersons. All symposia and workshops assembled in the programme are to be published in the 2nd Announcement of the Congress.
Summer–Fall 1988	Invitations are sent to chairpersons and speakers of symposia and to chairpersons of workshops. Invitations are sent for 'History of Immunology' and for presentations of 'Immunology in Brazil, The People's Republic of China, Hungary and the USSR'. The 2nd Announcement is mailed.
February 1989	Final assignments of locations and times for symposia and workshops are made, after poll of the advisers and submission of abstracts of participants.

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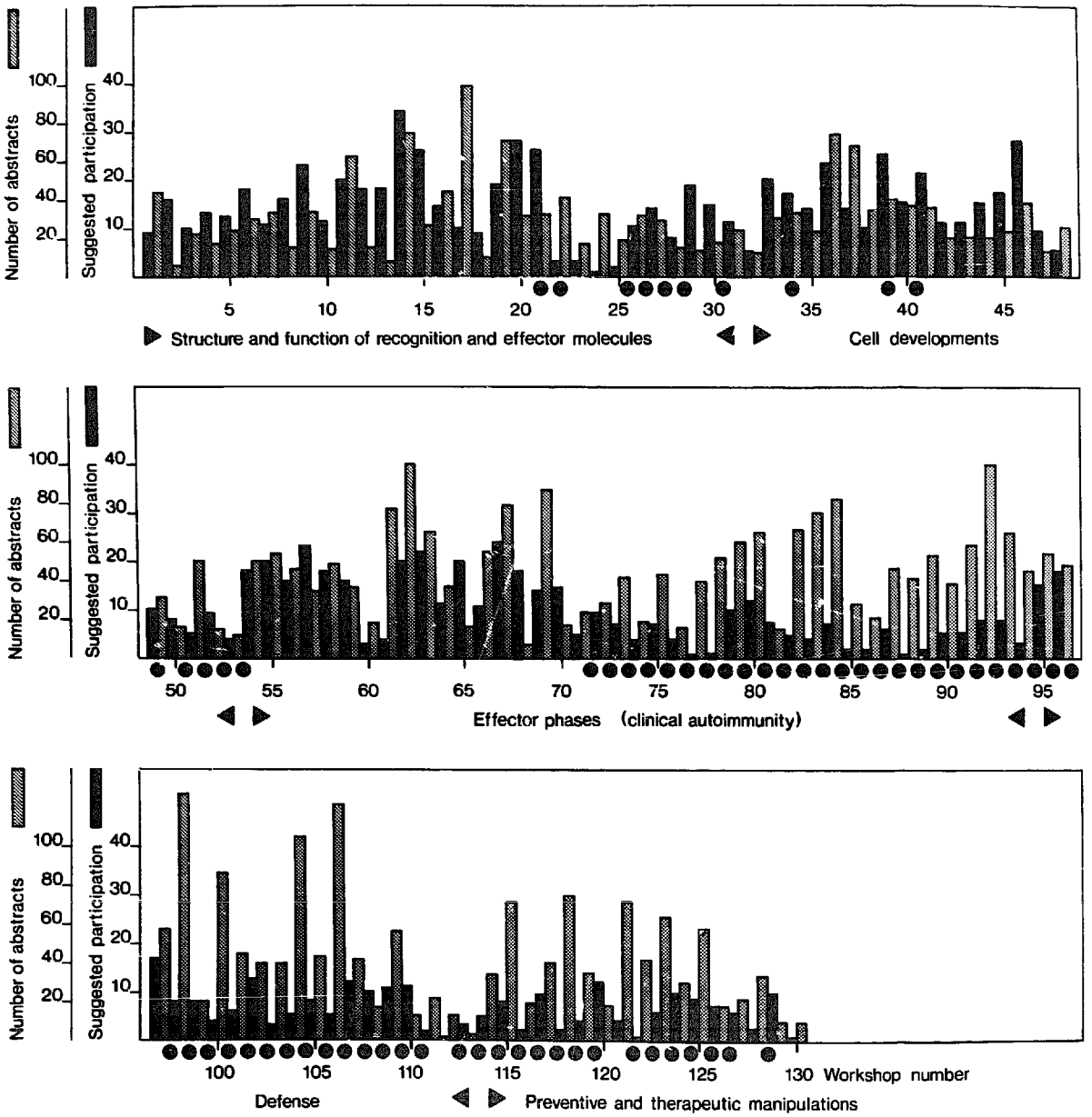

Fig. 1. *Participation in the 130 workshops of the congress is shown by the number of abstracts submitted (dotted bars). Based on the provisional program of the symposia and workshops, 140 national and international advisers to the program committee were asked to select the sessions that they would like to attend (closed bars). The horizontal axis indicates the workshop number: on the top left side (low numbers) are workshops with more basic interests, and on the bottom right side (high numbers) are those with more clinical and applied interests. The solid dots below the bars show workshops that the advisers considered to be of clinical interest. (However, of the abstracts actually received, many more covered clinically related topics than the advisers to the congress had anticipated.)*

Nevertheless, everyone realizes that, with the rapid advancements of modern immunological research, the program should provide some flexibility for the latest, hot news. Therefore, at least 30 minutes of each symposium have been kept open. The symposium chairpersons have been asked to suggest their latest additions to the program as late as possible, not earlier than four weeks before, and hopefully, only at the time when the congress convenes in Berlin. The latest, hot additions will be specially announced on each day of the congress.

Workshops often tend to be neglected. The program committee has tried to strengthen the role of the workshops within the total program as places not only for presentation of experimental results and ideas, but also for discussion. Half of the allotted time, therefore, should be devoted to discussions. Furthermore, the workshop chairpersons have been encouraged actively to solicit participation in the workshops from colleagues whom they consider important in their field.

Immunology is a fast moving, exciting field of science. The oldest participant of the 7th International

Congress, an honorary member of the Gesellschaft für Immunologie, Michael Heidelberger, was born when antibodies were discovered in Berlin. The youngest participant was not born when lymphocytes were discovered, when variable regions of antibodies were found, or when it was recognized that T and B lymphocytes had to collaborate to elicit an antibody response. The lunchtime sessions on the history of immunology will attempt to retrace some of these milestones in the discovery of the immune system. Also at lunchtime, four national societies will begin what the organizing committee hopes will be continued with other IUIS member societies in the future; the presentation of the activities of immunological research in Brazil, Hungary, the People's Republic of China and the USSR.

Even the most widely discussed program of the congress is likely to miss some important areas of the field, or must exclude in its process of selection promising talent and topics and even established investigators and fields. As chairman of the program committee, I sincerely apologize to all those who feel left out, not appreciated, undiscovered or forgotten. Some of the weaknesses were visible to the program committee even before the congress started: perhaps we could have attracted more scientists from the Eastern Bloc and from Third World countries – half of all the abstracts are submitted by scientists from the USA, West Germany, Japan and the UK; and only 7% of all symposium speakers and 16% of all workshop chairpersons are women.

One main aim of the program committee was to combine the interests of basic with those of clinical immunology. Will it work? The 5142 abstracts received for presentation at the congress, show, at least, that both areas are strongly represented, if not well balanced – much to the surprise of most of the national and international advisers (see Fig. 1). The program committee hopes that all of the many interests in the immune system will truly meet in Berlin, that the congress will contribute with exciting new discoveries to a better understanding of the immune system, and that many contacts will be made for successful international co-operation in the future.

The Basel Institute for Immunology, funded and supported by F. Hoffmann-La Roche Ltd Co., Basel, Switzerland, has been the site of the Scientific Secretariat of the Congress. My heartfelt thanks go to Ms Leslie Nicklin who, as secretary of the program committee, has helped organize the planning meetings and has handled the correspondence with nearly 100 national and 200 international advisers (altogether more than 15 feet of files, letters and records), and is now preparing the 5142 abstracts and approximately 250 contributions to the symposia and lunchtime presentations for the final program. Debbie Middleton's meticulous work is also gratefully acknowledged. On behalf of the organizing committee and the program committee, I wish to thank the Senate of Berlin-West, the German Research Council (DFG) and Boehringer Mannheim for their support of the meeting on "The Immune System in 1988" that was held in April 1988 at Schloss Elmau, FRG. Finally, I thank all my colleagues of the national and international advisory board for their co-operation, suggestions, criticisms and help in assembling the program.

Gesellschaft für Immunologie

J. Kalden[1], H. Kirchner[2] and H.G. Schwick[3]

The 'Gesellschaft für Immunologie' (GFI) was founded in 1967 by 19 investigators in Marburg. The founders decided against using the name 'German Society of Immunology' so that they could include members from German speaking countries in mid-Europe other than the Federal Republic of Germany. The first board of officers consisted of N. Hilschmann, K. Rajewski, H.G. Schwick and O. Westphal. It is interesting that the International Union of Immunological Societies (IUIS) was founded only two years later and that two of our officers (Schwick and Hilschmann) were also among its founders. The opinion of the founding members of the GFI was that, as a branch of natural sciences, modern immunology needed an organization for its representation as a professional group, and that the most important specific goals of the society should be the promotion of basic immunological research and support of the younger generation of scientists.

The first annual congress of the GFI was held in 1969 in Freiburg and attracted 315 participants. Since then, the GFI has held its annual meetings in the fall and, in fact, this year, because of the World Congress, is the first exception to this tradition. The meeting has been held several times in Austria and Switzerland, and twice a joint meeting has been held with the French society, in Strasbourg. Five years ago the meeting of the Leucocyte Culture Conference (the first meeting of which was actually held prior to the first GFI conference) was established as an additional spring meeting of the society, and it is particularly suitable as a forum for younger scientists to present their most recent data but has proven useful to all of us.

Table 1. Board of GFI in 1989

Board of GFI	Advisory Board of GFI
President: J.R. Kalden	T. Diamantstein
	K. Eichmann
Secretary: H. Kirchner	D. Gemsa
O. Götze	A. Reske-Kunz
K. Resch	G. Riethmüller
G. Wick	H.G. Schwick
	H. Wagner

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Table 2. Honorary members of GFI

1973	Hans Schmidt (1882–1975) Michael Heidelberger
1983	Otto Westphal
1986	Paul Kallos (1902–1988) Paul Klein

Since its foundation, the GFI has had three presidents (Westphal, Rother and Kalden) and two treasurers (Schwick and Kirchner). Its complete board consists of 12 officers (present composition shown in Table 1). At the time of writing the society has 973 members; the membership list is updated every second year and is currently being prepared for the 1989 issue. Together with the American, British, French and Japanese societies, the GFI is one of the five largest members of IUIS. The society has five honorary members (Table 2), and the Avery-Landsteiner award is biannually presented to an outstanding immunologist regardless of nationality (Table 3). This is the major prize of the GFI, which is kindly sponsored by the Behringwerke, Marburg. An additional prize, the Otto Westphal prize, is awarded every second year for an outstanding doctoral thesis.

The GFI is especially honoured to have been chosen to host the 7th International Congress of Immunology in Berlin and in accepting this honour, we are cognizant of our past and the terrible fate that many excellent scientists suffered at the hands of the Nazi regime. It was due to their loss that science, and immunology in particular, was slow to recover and grow in post-war Germany.

When the society was founded, only a handful of immunology research institutes existed in Germany. Among these it is probably fair to single out the Max-Planck-Institut in Freiburg (formerly headed by O. Westphal and the late H. Fischer). Many of the immunologists now active in Germany received their training in Freiburg. Many others went to outstanding research institutes abroad including Australia, Israel, Sweden, the UK and the USA. We are most grateful for the acceptance and the friendship we found, and for the scientific training we received in these countries.

Although there are many chairs of immunology at medical and science faculties, as well as other immunological research groups at various types of institutes (for example Max-Planck Society, Fraunhofer Society, industry, German Cancer Research Center) many university faculties do not have a department of immunology, and not all universities offer the opportunity of preparing a thesis in immunology. In addition, immunology is not appropriately included in the curriculum of medical students and students of biology. We cannot, therefore, afford to be complacent. However, with the World Congress in our country we may gain considerable support for the creation of better training facilities for young immunologists and improved professional opportunities for the more advanced scientists.

Table 3. Awardees of the Avery-Landsteiner Prize

1973	W.F. Goebel	USA
	J. Oudin	France
1975	H.G. Kunkel	USA
1977	K. Rajewski	Germany
1979	C. Milstein	UK
1981	S. Tonegawa	Japan
1983	I. Gresser	France
1985	P. Perlmann	Sweden
1987	J.J. Oppenheim	USA

Clinical research in the Federal Republic of Germany

Clemens Sorg and Joachim R. Kalden

During the second half of the nineteenth and the first two decades of the twentieth century, German clinical research enjoyed a good international reputation; this resulted from both the integration of scientific methodology into the field of clinical medicine and the original way in which the university hospitals were organized. These flourishing times of clinical research came to an abrupt end in 1933, when about five hundred medical professors had to leave the country – representing roughly 40% of the 1165 professors teaching at German universities in 1931. This exodus of expertise led to the almost complete loss of many medical disciplines, including the area of clinical immunology.

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Starting again in the early 1960s, it has taken more than twenty years to re-establish clinical research at university medical schools. At the moment, the Federal Republic of Germany has 27 medical faculties, comprising 1725 professors, assisted by a scientific staff of approximately 12 600, who teach more than 12 000 students per annum. (In comparison, the UK trains 4000 and the USA 17 000 medical students per annum.) In addition to teaching, however, German professors at medical schools spend a considerable part of their time on patient care, since the university hospitals are part of the general health care system and predominantly provide routine services to the community.

The training and education of medical students is not entirely satisfactory: during their studies, students are not exposed to research activities, either in the laboratory or in seminars, or in journal clubs. There is no mechanism at present to identify, encourage and train students showing particular scientific talents. A higher standard for doctoral

theses and for Habilitation (that is, the entry qualification for a later professorship) in the field of medicine also seems necessary. Such doctoral theses should perhaps be obligatory and have similar standards to those of the faculties of basic science.

With regard to immunology, most medical faculties offer courses and lectures in basic and clinical immunology but, since these subjects are not part of final examinations, only students with a particular interest in this area attend these courses. Efforts have repeatedly been made by the Gesellschaft für Immunologie to include basic and clinical immunology as part of the main teaching courses for medical studies, but so far they have been unsuccessful.

Once qualified, scientifically oriented young doctors face the problem that positions in university hospitals do not necessarily provide them with the freedom to perform research in addition to their clinical duties. This lack of opportunity to become actively involved in clinical research is well recognized and is one of the many reasons why young medical doctors seek further training abroad – particularly in the UK and the USA. A problem then arises when these doctors come home to 'nest' at German University hospitals that often cannot offer a suitable position to clinically oriented scientists.

However, new efforts to improve the structure of clinical research in the Federal Republic of Germany have begun recently, which were initiated by the major science organizations, especially the Wissenschaftsrat. The Bundesministerium für Forschung und Technologie (Ministry for Research and Technology), the Deutsche Forschungsgemeinschaft and the Max-Planck-Gesellschaft have also initiated programs to improve clinical science. Thus, the institution of so-called 'Sonderforschungsbereiche' – that is programs concentrating on a particular university research interest in one major area – are conducted and controlled by the Deutsche Forschungsgemeinschaft. Similarly, Forschergruppen at university levels or clinical research groups have been established by the Max-Planck-Gesellschaft, the Deutsche Forschungsgemeinschaft and the Bundesministerium für Forschung und Technologie. These clinical research groups are similar to the MRC Clinical Research Units – although smaller – and have been developed successfully at the Universities of Munster, Gieben, Wurzburg, Gottingen, Erlangen and Freiburg. In addition, the Bundesministerium für Forschung und Technologie has established a research program called 'Forschung im Dienste der Gesundheit', which concentrates on basic and clinical research in the fields of allergy, cancer, pulmonary diseases, AIDS, infectious diseases, cardiovascular diseases, rheumatic diseases and autoimmunity.

To make further progress, the fundamental structure of the German universities needs to be analyzed. German university professors, including those at medical faculties, enjoy tenured positions from their first appointment; this makes it difficult to develop procedures for redistribution of resources according to efficiency and achievements. However, looking back at the development of clinical research in this country over the past twenty years, it can be seen that considerable progress has been made, mainly due to programs initiated and conducted by the major science organizations. The present situation should be maintained and further improved. All societies in Germany engaged in clinical research should continue to support the Wissenschaftsrat, the Forschungsgemeinschaft, the Max-Planck-Gesellschaft and the Bundesministerium für Forschung und Technologie. This concerted effort will then further improve clinical science in our country.

Further reading

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Raising funds for basic science in the FRG – no special deal for immunologists

Matthias Cramer

The Federal Republic of Germany (FRG) spends 3.8% of its gross national product on science[1]. It is debated whether or not this is enough for a wealthy country with scarce natural resources but an impressive economic power. The total sum of money spent for basic science in the FRG in 1987 was calculated to be 7.8×10^{10} DM (Ref. 1) – 55% of which (4.3×10^{10} DM) stems from fiscal and non-profit orientated institutions[1].

In this article, we will restrict discussion to the situation of immunology as a basic science in the FRG. Clinical immunology is dealt with in a separate section.

It is clear that immunology in the FRG is far from being an established scientific subject in its own right. In the vast majority of German universities (and their medical schools) immunology is dealt with under headings such as microbiology, virology, oncology, genetics, molecular biology, experimental medicine, biochemistry or cell biology. While the number of chairs for subjects such as immunology, immunobiology and molecular immunology is slowly increasing, a degree in immunology can be obtained in only a few places. Although the spreading of immunology into these many fields is a terrific source of progress for our scientific endeavours, it renders any attempt to quantify the amount of

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money spent on immunology in the FRG impossible.

The need for funding – the structure of research institutions in the FRG

In order to understand the German system of funding research, one needs to understand the organization of the existing (and competing) research institutions in this country.

First, there are the universities. All German universities are run by the state. Within the framework of the federal organization of the FRG, basic science (universities) is the responsibility not of the federal government (in Bonn), but of the eleven States (Bundesländer) and their state governments (in, for example, Düsseldorf, Munich, Stuttgart and Hannover). Thus, universities in the various states have slightly different organizational structures and certainly different financial problems. The universities provide their research institutes with personnel, buildings, basic equipment and instrumentation, and infrastructure, requiring heavy investment. Financing an internationally competitive immunological research group is clearly beyond the scope of any standard German university, however, and research in university groups needs additional funding.

Second, there is the Max-Planck-Gesellschaft (MPG) and the Max-Planck-Instituts. These focus on natural sciences including immunobiology, immunogenetics and immunochemistry. The principal source of MPG's money is the Federal Government. In contrast to the universities, the MPG can provide funds to fully support the research of its groups. Full support is a relative term, however, and most immunological groups at Max-Planck-Instituts also apply for additional money.

Third, there are a number of special federal research centers (Großforschungseinrichtungen), among them the German Cancer Research Center (DKFZ) in Heidelberg. Despite their academic freedom these centers have a broad but defined scientific topic to work on. These centers also have 'hard' money, but again, additional funding is sought.

Finally, there are exceptions from and mixtures of these three institutionalized concepts – for example, Max-Planck groups not solely financed by the MPG, or university institutes not in the university but associated with it.

The Deutsche Forschungsgemeinschaft (DFG) – the main agency funding basic research

The Deutsche Forschungsgemeinschaft (DFG, German Research Agency) in Bonn is an independent, federal institution. Its purpose is to fund all aspects of basic science in the FRG[2,3]. The DFG has a total annual budget of 1.1×10^9 DM (Refs 1, 2). As part of the federal burden-sharing, the DFG expects the universities/MPG to provide all the previously described academic and other infrastructures.

The researcher/immunologist has three major routes to apply for DFG money. First, she/he may apply for an individual grant for an individual project (Normalverfahren). This may include personnel, instrumentation and running costs for scientific equipment. These grants run for one or two years and may be prolonged. The DFG spent 4.4×10^8 DM on this program last year, 1.6×10^8 DM of this on biomedical sciences. Second, a group of scientists working on a related subject but who are distributed around the country may get together and apply for a common, topic-oriented grant, called a Schwerpunkt, within which the members have their individual projects. One period of funding runs for two years. Last year the DFG spent 2.1×10^8 DM on this program, 6.1×10^7 DM of this for biomedical science. Finally, an already existing, active research center such as a combination of research institutes within one university or town may establish a Sonderforschungsbereich (SFB, special collaborative grant) with a scientific heading to which all projects must be related. SFB grants are reviewed every three years. The SFB program has 3.2×10^8 DM, 1.2×10^8 DM of which are for biomedically oriented SFBs.

In principle, the access to DFG money is mediated via the scientific standard of the applications only. The DFG developed a peer reviewing system, and the rejection rate of applications may reach 15–30%. On a statistical basis, only half of the money applied for is actually allotted to the applicant's project by the reviewers.

Other state and private institutions

The Federal Minister of Science and Technology (Bundesminister für Forschung und Technologie, BMFT) finances applied science developing into technology. Since the borders between basic and applied science are sometimes questionable, a substantial amount of the BMFT money also goes into aspects of basic immunology.

The various states (Bundesländer) either by themselves or in conjunction with federal sources, also put up programs to foster research institutions. The form, extent, focus and duration of these efforts are different in every case, since they are expected to solve different questions.

Finally, we should turn to the sector of private funds and foundations supporting basic science/immunology. A number of foundations have joined into the Stifterverband für die Deutsche Wissenschaft, which coordinates private funding at various levels[4]. Numerous foundations do not operate under this cover, however and it is not possible to list them all in this article. In general, the support by private sources is a special deal between the institution, the researcher and the foundation. Since these arrangements are tailor-made, the advantages of private funding are much greater than the actual amount of money spent may suggest.

This article contains a number of crude oversimplifications, and ends with a remark which may not be shared by all our colleagues around the country: that with good immunological science one may raise funds in the FRG. The national funding of basic science is open, critical, balanced and fair. This is verified by the fact that working on international (or European) money is the exception rather than the rule in the FRG.

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THEME ONE

The following three articles have separate authors but share a common theme and have been united under a general heading.

Ig genes, molecules and B-cell development

Immunoglobulin genes

Hans G. Zachau

The immunoglobulin gene alphabet was first spelled across the border from Germany, in Basel, Switzerland, by Susumu Tonegawa and his colleagues: *V* (variable), *J* (joining) and *C* (constant) gene segments. Work from laboratories all over the world contributed to this and to the rest of the alphabet – the *D* (diversity) and *N* (nucleotide insertion) elements.

The current state of research on immunoglobulin genes is described in numerous review articles and in two books, one in 1987 (Ref. 1) and one which is due to appear now[2]. This article will not be a comprehensive national review, but will mention the work of a few German laboratories that have contributed to the field recently.

The organization and various functional aspects of the mouse heavy chain locus have been studied in K. Rajewsky's department in Cologne. The mechanism of isotype switching is currently being studied by A. Radbruch and colleagues; recently they investigated the interleukin 4 (IL-4)-induced transcription in the target switch region which seems to be a prerequisite of directed switch recombination[3]. Some time ago, R. Dildrop established a new classification of V_H chain sequences[4] which agrees fairly well with the results of V_H gene mapping. The latter topic is being studied by U. Krawinkel's group, now in Freiburg. By a combination of genetics and blot hybridization they contributed to the present picture of the V_H locus as being organized in overlapping clusters of the known gene families[5].

To my knowledge, no systematic gene cloning and chromosomal walking studies on the mouse heavy chain or κ loci have been performed to date. Only the comparatively simple λ locus that contributes 5% of the light chains has been studied extensively. The reverse seems to be true in the human system: little is known about the λ locus while the elucidation of the heavy chain locus is well under way in at least two laboratories[6,7]. Following the initial work by Bentley and Rabbitts[8], the κ locus has been studied by our group in Munich for the past few years.

Our work started with the κ genes of the mouse: *V-J* joining and its reciprocal recombination products[9], allelic exclusion, somatic mutations, promoters and expression[10] and gradually ascended to the κ genes of man. To date, we have cloned about 70 $V_κ$ genes and linked them in six contiguous segments; however many of the genes were pseudogenes. The total number of $V_κ$ genes and pseudogenes will probably be well under 100. The map of the locus as delineated by pulsed field gel electrophoresis covers about 2 megabases (Mb) (Fig. 1). It can be predicted on the basis of the transcriptional polarities indicated in the map that different *V* genes rearrange to the *J–C* gene region by different mechanisms: the two *J* proximal *V*s by inversion, the subsequent ones by deletion; the *V* genes of the 0.85 Mb NotI fragment should be rearranged by inversion since the reciprocal recombination products are found in the genome.

A unique feature of the $V_κ$ genes of man is the existence of numerous non-processed genes outside the locus, on the same chromosome (Fig. 1) and on other chromosomes[12]. We cloned about two dozen such $V_κ$ orphans and found some to be rather divergent but most of them only slightly defective functional $V_κ$ genes.

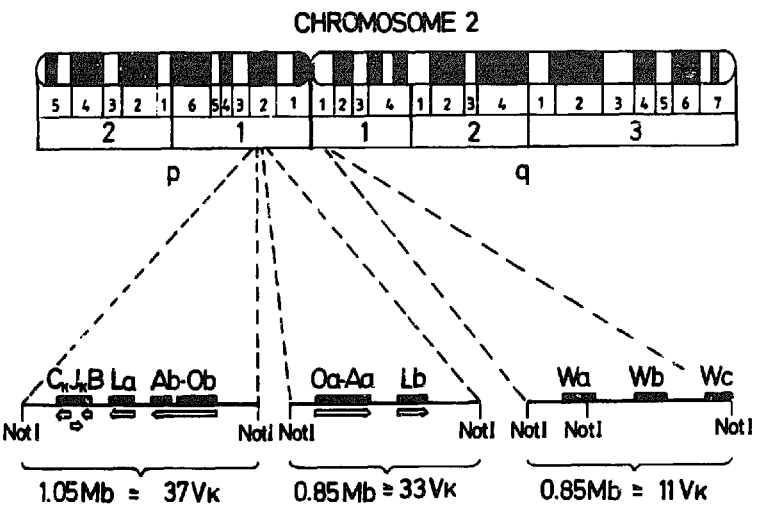

Fig. 1. *The human κ locus on chromosome 2 at 2p1 (Ref. 11; G. Weichhold, unpublished) and of the orphaned W regions at 2q1 (F-J. Zimmer, H. Hameister, R. Thiebe and H.G. Zachau, unpublished). Thick bars indicate cloned regions and open arrows the transcriptional polarities of the gene segments.*

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Chromosomal translocations of the immunoglobulin loci to the *myc* oncogene region, for example, as found in Burkitt's lymphoma, have been studied for several years in G. Bornkamm's laboratory[13] and more recently also by M. Lipp and colleagues[14]. Our group concentrated on the detailed characterization of breakpoints within the κ locus[15].

Immunoglobulin gene expression is a booming business worldwide. While mechanistic aspects are pursued mostly by molecular biologists, the immunologist's interest is in the production of functional antibodies or antibody fragments from specifically designed genes. The work of two groups should be mentioned in this context. A group at Penzberg (Boehringer Mannheim) started by cloning from a hybridoma and expressing the genes for an antibody against creatine kinase[16]. Their main aim is to produce antibodies of diagnostic and therapeutic value. A. Plückthun's group in Munich succeeded in producing an 'antibody' in *E. coli* that consists of just the variable domains but binds phosphorylcholine well[17]. Their objective is largely mechanistic and concerns problems such as the folding and assembly of chains and ligand binding.

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Somatic antibody mutants

Klaus Rajewsky

The hypothesis that somatic mutation contributes to antibody diversity has been disputed by immunologists for a long time and on occasions bitterly. Strong support for the hypothesis came from early work of Weigert and colleagues on the primary structure of murine λ1 chains[1]. However, direct evidence for somatic mutation in antibody V regions required molecular biology techniques that allowed the comparison of the sequences of V regions carried in the germ line with those expressed by somatic cells (reviewed in Refs 2 and 3). An early case of localized somatic hypermutation was reported by Zachau's group in Munich, who compared the productively and non-productively rearranged V_κ genes of a myeloma to their germ line counterparts and found multiple somatic point mutations in both[4]. The same group has provided further examples of somatic mutation in V_κ genes in the course of their work on the V_κ gene cluster in humans (see Zachau's contribution in this issue).

In terms of the physiology of the immune system, the central question is to what extent and in what way somatic mutation contributes to the immune response. The development of monoclonal antibodies[5] provided a means of addressing this problem. In 1981, the first sequences of *V* region genes expressed in murine antibodies isolated from different stages of the immune response were obtained and compared to the corresponding germ line genes; this included an experiment that we carried out in collaboration with Baltimore's group[6,7]. The results showed that somatic antibody mutants are selected in the response to antigen. It soon became clear through studies in a variety of models (see Ref. 3 for review) that as a rule, primary responses consist of germ-line-encoded antibodies, whereas in secondary and hyperimmune responses to T-cell-dependent antigens somatically mutated antibodies are expressed. These mutants arise through a special hypermutation mechanism which introduces point mutations into rearranged *V* region genes at a high rate – higher even than the rate of approximately 10^{-5} per base pair per generation as determined by Wabl *et al.*[8] in a pre-B-cell line. Gene conversion – identified as a major mechanism of antibody diversification in the chicken[9] – may play some[10,11], but not a major role in the generation of somatic antibody mutants in the mouse.

The analysis of the pattern of somatic mutation in clonally related B cells showed that the mutations are introduced in a stepwise manner in the course of clonal growth[12–14]. Somatic hypermutation does not necessarily accompany B-cell proliferation (we have not seen it, for example, in expanding Ly-1$^+$ B-cell clones[15]), but seems restricted to a particular pathway of B-cell differentiation, namely the generation of antigen-driven, T-cell-dependent B-cell memory; there is evidence that the process of mutant generation is set in motion by immunization[16–17]. Somatic antibody mutants are then selected into the compartment of memory B cells, and, as shown by Berek *et al.*[18], clones of such cells may persist in the animal for a long time. Upon a second contact with

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antigen, the mutated cells are able to expand and terminally differentiate into plasma cells in the absence of somatic hypermutation, as we have shown by analysis of clonally related cells isolated from an adoptive secondary response[19]. However, it is possible that some cells again enter the pathway of somatic hypermutation following a secondary stimulus[20]. C. Berek, who now heads a research group at the Genetics Institute at the University of Cologne, attempts to clarify this matter and to determine the microenvironment in which hypermutation is induced – perhaps the germinal center (see Ref. 21).

The accumulation of somatic mutations in the antibody response coincides with affinity maturation – i.e. the selection of high affinity antibodies. There is direct evidence that the two processes are causally related (reviewed in Ref. 3). Thus, in the case of the response to the hapten 4-hydroxy-3-nitro-phenylacetyl we found that a single mutation, repeatedly seen in the same position, contributed most of the affinity increase observed in the antibody population. The other mutations carried by the antibodies apparently accumulated as 'background noise' because of the high rate of somatic mutation[22,23]. In contrast, in the response to a (more complex) protein antigen, affinity maturation occurs in a stepwise way, concomitant with the stepwise occurrence of somatic mutation in the course of clonal expansion[24].

Thus, in a particular and critical pathway of acquired immunity (namely that of memory generation in the B-cell compartment) a new repertoire of antibodies is generated through a special mechanism of somatic hypermutation and cells expressing mutated antibodies are selected as memory cells on the basis of their affinity for antigen. This process results in affinity maturation of the antibody response. It may also enable the immune system to cope with microbial mutants arising in the course of infection.

The physiological risk of somatic hypermutation lies in its potential to generate autoreactivity. Model experiments from our laboratory[25,26] and by others (see Ref. 3) have shown that single point mutations can result in profound changes of antibody specificity, and there is also evidence that antigen binding loss mutants are generated after immunization[19]. Mutants of this type may occasionally have acquired specificity for a self antigen[27] and produce autoimmune disease[28]. The mechanism of somatic hypermutation and its (T-cell mediated?) control is thus a key issue for future research.

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Allelic exclusion – one facet of B-cell development

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An important step in B-cell development is the assembly of spatially separated gene segments into contiguous immunoglobulin (Ig) heavy and light chain variable region (V_H and V_L) genes. First V_H and then V_L genes are rearranged and this leads to the sequential expression of H and L chains in pre-B cells. The regulation of the rearrangement process is an interesting area of B-cell research which is actively pursued in laboratories in Cologne and Freiburg. This short article discusses experiments covering this facet of B-cell development.

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Two experimental systems have been used to study the regulation of rearrangement. First, the B-cell compartment of mice transgenic for fully rearranged H and L chain genes was analysed. Second, H and L chain genes were introduced into actively rearranging pre-B-cell lines and their effect on rearrangement was studied. The results of these studies are summarized in Fig. 1.

Mice expressing transgenic μ

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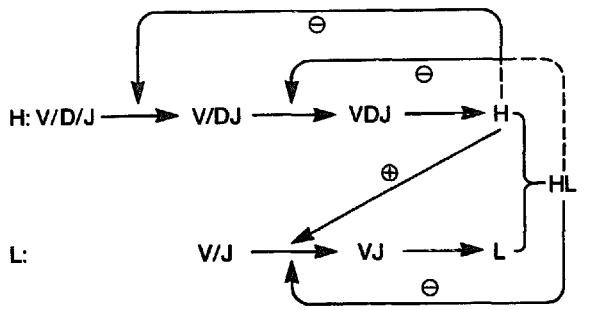

Fig. 1. *Regulation of the assembly of immunoglobulin heavy (H) and light (L) chain variable region gene segments. From pools of several hundred $V_H(V_L)$ segments, 12D_H and 4$J_H(J_L)$ segments $VDJ_H(VJ_L)$ complexes are assembled and functional H and L chains are expressed[1]. The H and L chain products regulate the rearrangement of the respective gene-elements thus ensuring the functional assembly of only one H or L allele, a phenomenon known as allelic exclusion[2].*

heavy chains have fewer B cells, most of which express the transgene and only a few coexpress endogenous H chains[3–5]. The lack of endogenous H chain expression is because the endogenous Ig H alleles remain unrearranged or in an immature *D* to J_H joining configuration, supporting the hypothesis[6] of a negative feedback of the H chain on its own rearrangement (see negatively marked arrows in Fig. 1). Refinement came from experiments showing that only the membrane anchored μ and not its secreted form (lacking the transmembrane region) exerted the negative feedback[7,8]. It is debated whether the H chain alone, the H–L-chain combination or both are functional in the negative feedback inhibition (see broken arrows in Fig. 1).

Arrest of endogenous V_H gene rearrangement in transgenic mice may be due to an accelerating effect on B-cell differentiation. Since the trans-μ is expressed earlier in ontogeny than endogenous μ (Ref. 4), B-cell development could proceed more quickly to V_L-rearrangement leaving fewer pre-B cells and less time for endogenous V_H rearrangement. This hypothesis could explain why in these mice, Abelson-Murine-Leukemia-virus (Abl-MuLV) transformation of pre-B cells is about 100-fold less efficient (Ref. 9; G. Köhler, unpublished). Alternatively, a direct effect of μ in shutting down further V_H rearrangements is possible. Such a negative feedback has been observed by M. Reth (Cologne) and collaborators using the Abl-MuLV transformed pre-B-cell line 300-19 (Refs 10,11). This line carries a DJ_H3 join on each Ig H allele and continues to rearrange its V_H locus while growing in culture: 12 secondary *D* to J_H4 and 19 *V* to *DJ* rearrangements in 65 reclones were counted. When a construct coding for membrane μ (with or without a λ light chain) was introduced by DNA transfection techniques, only 2 *D* to J_H and no V_H to DJ_H joins were observed in 31 reclones analyzed which argues in favour of a direct negative effect of μ in the V_H-rearrangement process.

No V_L-gene rearrangement was observed in these clones. However, in subclones carrying nonfunctional VDJ_H rearrangements on both Ig H loci, expression of transfected membrane, but not secretory μ, led to the onset of V_L rearrangements[11]. It was concluded that in pre-B cells of a more mature phenotype, membrane μ induces V_L rearrangement (Fig. 1, positively marked arrow). Evidence for this came from transgenic mice in which the major B-cell population expressed transgenic H with endogenous L chains[4,7,12]. This indicated that the transgenic H chain did not inhibit and may have induced L chain expression.

The first evidence of Ig-feedback regulation on rearrangement came from κ transgenic mice[13]. In only those B cells expressing endogenous H chain together with the transgenic light chain was the expression of endogenous L chains suppressed. In these cases the endogenous V_L locus remained in the unrearranged germline configuration. It was concluded that the H–L chain combination, but neither chain alone inhibited further V_L rearrangement (Fig. 1, negatively marked arrow).

To learn more about which domains of the μ chain are required for its regulatory function and whether other Ig H chains were also effective, mice transgenic for the Ig δ chain were analysed. These mice showed a similar phenotype as μ transgenic mice[14]. Since the δ chain lacks the domains homologous to Cμ2 and Cμ3, it was concluded that these domains could be dispensable for the regulatory effects. In contrast, expression in mice or in a pre-B-cell line of a μ gene lacking the first constant domain did not activate V_L rearrangement (G. Köhler, unpublished). Light chains interact with the V_H domain, and covalently with the Cμ1 domain. They are necessary for the membrane deposition or secretion of μ. Presumably the light chain displaces the heavy chain from a protein (gp78) of the endoplasmic reticulum which binds to the first constant domain of heavy chains[15]. If, indeed, surface expression of heavy chains in pre-B cells is a prerequisite for their regulatory function, other proteins may also be required. This points to a role of genes specifically expressed in pre-B cells called $V_{pre\text{-}B}$ (Ref. 16) and λ5 (Ref. 17). They are homologous to the variable ($V_{pre\text{-}B}$) and the constant (λ 5) region of the λ light chain and their presumptive products are expressed in association with μ chains on the surface of some pre-B-cell lines[18,19]. In addition, genes (B29, MB-1) have recently been described, that are expressed in pre-B cells and B cells[20,21]. The product of one of these genes (MB-1) is apparently associated with the membrane μ chain and is necessary for its surface expression[22]. Furthermore, an amino acid sequence motif has been identified in the cytoplasmic portion of B29 and MB-1, which is shared by proteins forming the Fcε receptor and by members of the T3-complex[23]. The proteins of this complex are associated with the T-cell receptor and are important for its surface expression. They may also be necessary for signal transduction during antigen activation. It is conceivable that a B3-complex, with similar functions, exists for the B-cell immunoglobulin receptor and that it also plays a role in regulating early B-cell differentiation.

During these studies other important discoveries have been made. The

V-replacement recombination into fully rearranged V_H loci has been found, which increases H chain diversity and possibly decreases the number of nonfunctional rearrangements[24,25]. The progression to λ chain expression seen in a pre-B-cell line apparently depends on a prior recombination event involving downstream recombining sequences and resulting in deletion of both κ alleles[26]. Finally, evidence that suggests why allelic exclusion must be tightly controlled has been put forward[4]. Transgenic mice coexpressing trans- and endogenous Ig on the B-cell membrane are unable to respond to T-cell-independent antigens of the class II type, but do respond almost normally to helper T-cell-dependent antigens. Specific serum IgM of T-cell-dependent responses consisted of mixed molecules of endogenous and transgenic origin, whereas serum IgM of T-cell-independent responses did not show the transgenic component. Possibly only those rare cells that have lost the transgene expression were able to respond in a helper T-cell-independent fashion.

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H-2 genes and function

Günter J. Hämmerling and Bernd Arnold

In Germany, research on murine histocompatibility (H-2) antigens shows a satisfying degree of polymorphism with each group working in this field going through minor or major evolutionary changes. There are two main centers of H-2 research, Tübingen and Heidelberg, and many small groups distributed throughout the Federal Republic of Germany. Recognizing the importance of this field, major histocompatibility complex (MHC) research was supported for six years by a special programme (Schwerpunkt) from the major German funding agency, the Deutsche Forschungsgemeinschaft. This support was extremely valuable and led to many interactions between the German laboratories involved. However, the latest philosophy of the Deutsche Forschungsgemeinschaft – that it is sufficient to teach a young bird to fly – means that funds are now going preferentially to 'new and innovative projects', unfortunately disregarding the fact that established fields can also develop new and innovative directions which require funding.

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When writing about MHC research in Germany one must mention first the illustrious and sometimes controversial figure of Jan Klein, who resides in the Max-Planck-Institut for Biology in Tübingen, secluded in the pleasant mountains of the Schwäbische Alb. Perhaps too secluded it is felt, with regret, by many of his German colleagues. He was one of the founders of the rapidly expanding field of MHC research and has had a considerable impact on many of us from the days when he and Donald Shreffler proposed the two locus model of the H-2 complex in 1971 (Ref. 1). Jan Klein is a real scholar, who has given us important conceptual frameworks in MHC immunogenetics, and has always tried to bring order into the MHC and its nomenclature. Besides a vast number of papers, he has also written several important textbooks in which he does not hesitate to express his personal opinion, usually in a refreshing historical perspective. His *Natural History of the MHC Complex*[2] is excellent – delightful to read and an endless source of information. His witty and cutting commentaries in the 'Odd Page' of his journal *Immunogenetics* are also delightful to read. For example, his glossary entitled "They don't cite me"[3] expresses nicely the feelings and difficulties faced by most of the authors of this issue of *Immunology Today* who, in a limited space, are trying to present a fair picture of immunology in Germany.

MHC polymorphism

Jan Klein's major interest was, and is, the polymorphism and evolution of the MHC complex. In a study on wild mice captured all over the world, he and his group found that

for each of the class I and class II loci there are 60 to 100 or more alleles. Until recently, all cases of polymorphism were thought to have arisen after speciation. However, in many papers Jan Klein has provided more and more conclusive evidence that MHC polymorphism pre-dated speciation[4]. This novel hypothesis was voiced by him as early as 1980. Studying wild mice, Klein also observed a surprisingly high frequency of lethal or semilethal t haplotypes. He proposed that the various t chromosomes were derived from a single ancestral chromosome. Jan Klein is not someone who avoids controversies. When he and Zoltan Nagy observed that liposomes can stimulate class-II-restricted T-cell clones, he proposed that antigen processing was not always necessary – a conclusion that is disputed by other investigators. During their studies on lactate dehydrogenase (LDH-B)-specific and I-E-restricted, suppressor T cells and suppressor factors, Klein and Nagy suddenly found themselves on 'an involuntary trip'[5] into the dubious world of I-J antigens which they had previously fervently erased from the H-2 map.

Another group studying MHC polymorphism is that of E. Günther in Göttingen. Using serology and cDNA cloning, Günther investigated rat MHC[6,7]. Only one class I antigen is known to act as a restriction molecule (RT1-A) for cytotoxic T lymphocyte (CTL) recognition. Next to the RT1-A locus are human leukocyte antigen (HLA) DR-, DQ- and DP-like class II genes, class III (complement) and another class I cluster, probably harbouring Qa-like genes. The degree of polymorphism within the rat MHC appears to be much more limited than the H-2 complex, with only a dozen class I alleles for RT1-A locus and altogether about ten class II alleles. However, in contrast to the mouse, only a few wild rats have been investigated, and such studies could reveal a more extensive polymorphism. Since the rat is a good model for organ transplantation, these data are useful to groups studying the influence of various RT1 gene products on experimental organ transplantation.

While many immunologists feel obliged to do something useful, the laboratory of Klaus Rajewsky in Cologne is one of the strongholds of intellectual immunology. Although primarily involved in other fields, due to his wide interest in immunology, Rajewsky could not leave the MHC untouched. Using the first monoclonal anti-H-2 antibodies, which were produced in his laboratory by Lemke and Hämmerling in 1977 (Ref. 8), B. Holtkamp and colleagues selected structural variants of the H-2K^k molecule[9]. With W. Weichel, they showed that the respective epitopes could be mapped to the outside of those parts of the class I molecule that form the peptide-binding groove. These structural variations had little influence on T-cell recognition, thus confirming the view that antibodies and T cells see different epitopes on MHC molecules.

MHC: structure–function relationship

After Günter J. Hämmerling's move from Cologne to the German Cancer Research Center in Heidelberg, the latter developed into one of the major centers for MHC research. Most studies were aimed at understanding the relationship between structure and function. The epitopes recognized by a large panel of monoclonal, anti-H-2 antibodies were mapped and found to be different from those recognized by CTL. More precise information was gained from the class I exon shuffles generated by Bernd Arnold and Sune Kvist (the latter working at the EMBL). These early studies showed that the class I determinants seen by CTL were exclusively formed by the α1 and α2 domains[10], which is consistent with the picture derived from Björkman's crystallographic structure of the HLA-A2 molecule. However, the α3 domain is also important because it is involved in the interaction between the Lyt-2 accessory molecule and H-2 class I (B. Arnold, U. Kalinke and G. J. Hämmerling in collaboration with A. Höveler, B. Malissen and A. Schmitt-Verhulst in Marseille). These authors also observed a species-specific barrier because in mouse T cells the human CD8 molecule could not take over the function of Lyt-2. Preliminary evidence suggests that this CD8/class I species barrier is also the explanation for the notoriously weak xenogeneic CTL responses in comparison with the powerful alloresponses. Modifying what Jan Klein has said about alloreactivity, this perception of xenoreactivity would be the 'crowning of research on one of the most intensively studied artefacts in immunology'.

Tumor expression of MHC antigens

Another major research area in Heidelberg is the role of MHC antigens on tumors. Many tumors in both rodents and humans have defective MHC class I expression. Hämmerling's group observed that the restoration of class I expression by class I gene transfection led to the abrogation of tumor growth and metastasis in syngeneic mice[11]. Subsequent studies showed that the transfected class I molecule provided the H-2 restriction element required for the recognition of a tumor antigen by CTL. While it is accepted that CTL require class-I-positive target cells in several systems, an inverse correlation between natural killer (NK) lysis and class I expression on the NK targets has been observed. In H-2 gene transfection studies, Sturmhöfel and Hämmerling showed that the expression of the class I antigens was directly responsible for the increased resistance to NK lysis. The importance of MHC antigens for tumor growth led to studies on the molecular regulation of MHC expression. U. Maschek has found that K and D genes can be silent because of changes in the chromatin structure. In contrast, for another tumor, D. Klar has identified a defect at the post-translational level. In these tumors H-2 and β_2-microglobulin coexist without assembly. However, assembly and cell surface expression can be induced by stimulation with gamma-interferon (IFN-γ) which indicates that cellular factors may be required for an efficient assembly[12].

To test whether the lack of HLA expression on human tumors correlated with more metastasis and higher malignancy, a large number of human tumors were screened in many laboratories. In Heidelberg, Momburg, Möller, Hämmerling and colleagues found deficient HLA expression in 30% of colorectal carcinoma specimens[13]. However, MHC had no influence on survival or relapses in a follow-up study. On the other hand, HLA-deficient human B-cell lymphomas fell predominantly into the more malignant group.

MHC transgenics

Recently, many class I and class II transgenic mice have been produced

in Heidelberg to study tolerance, immunity and MHC function. To investigate whether soluble MHC antigens could fulfill the function of membrane bound MHC, B. Arnold produced transgenic C57BL/6 mice secreting soluble H-2K^k into their serum[14]. These mice were not tolerant to membrane bound K^k because they could still make K^k-specific CTL and antibodies, possibly because soluble K^k cannot efficiently crosslink receptors or interact with Lyt-2. In all textbooks, the HLA-C antigens are attributed with the same functions as HLA-A and HLA-B although no experimental data existed to support this assumption. Using HLA-Cw3 transgenic mice, Dill and colleagues[15] first showed that HLA-C could indeed function as a transplantation antigen and as an MHC restriction element. The HLA transgenic mice have proved to be extremely useful. Because they are tolerant of HLA framework determinants, they are powerful tools for the production of monoclonal antibodies (mAbs) against polymorphic HLA determinants (Günter Hämmerling in Heidelberg, and Ulrich Hämmerling in New York, in collaboration with B. Dupont and S. Young, Sloan-Kettering Institute, and S. Weissman and J. Chamberlain, Yale). This new technique may change the field of HLA typing by setting it on a basis of standardized mAbs.

Qa region products

Peter Robinson (now in Harrow) spent many years in Heidelberg. As early as 1981 he wrote a cautionary note that the unexpected expression of 'alien' H-2 antigens on tumors is frequently best explained by a genetic impurity of the tumor cells or mice (a finding which was recently rediscovered for some UV-induced tumors). His main fields of interest were β_2-microglobulin (β_2m) and Qa region products. He described two allelic forms of β_2m in inbred mice and found further alleles in wild mice. He also showed that β_2m, which is associated with class I antigens on the cell surface, could be exchanged with exogenous β_2m. Since class I molecules bind β_2m, Robinson used anti-β_2m antibodies for identification of new Qa region encoded products. He found a class I protein called Qb-1, which was found to be secreted by lymphoid cells[16]. There are two pathways by which Qa antigens can be secreted[17]. For example, Qa-2 is membrane bound via a glycosylphospholipid anchor and can therefore be processed to a soluble form. Qb-1 on the other hand, has an incomplete membrane-associating domain. The Qb-1 antigen is encoded by the Q4 gene, as found by gene transfer studies by Robinson and Weiss[18]. Elisabeth Weiss (Munich) has analysed the organization and structure of the Qa region genes in the C57BL/6 mouse showing that the Q4 to Q9 genes possessed very similar structures. The corresponding region of the H-2^k haplotype contains only three genes: Q4, Q5 and Q10.

Class-II-associated invariant chains

N. Koch (Heidelberg) worked on the class-II-associated invariant chain (Ii) which, in general, is co-expressed with class II and can be co-induced *in vivo* in various tissues, with IFN-γ (Ref. 19). Transfection studies showed that one gene gives rise to two proteins, Ii31 and Ii41, due to differential RNA splicing[20,21]. The Ii sequence exhibits some similarities with the transferrin receptor – such as a fatty acylated cysteine close to the transmembrane region – and a sequence motif which is important for the recycling of the transferrin receptor. This suggests that the Ii chain may also be recycled. In this context it is of interest that a recent mAb detects Ii on the surface of human B cells (C. Wraight, G. Moldenhauer, P. Möller and P. van Endert). The membrane orientation of Ii is the opposite to that of class II, with the amino terminus in the lumen and the carboxy terminus on the outside. No cleavable leader sequence was found[22] that is characteristic of class II proteins. Dobberstein's group at the EMBL use the Ii chain in order to understand how such class II proteins are transported through the various cellular compartments. Since the Ii chain is found in the endosomal compartment, it is possible that it transports the class II antigens to this compartment, where the class II molecules could meet peptides of endocytosed and processed antigens. In fact, recent studies by Stockinger and Koch suggest that the presence of the Ii chain is necessary for efficient antigen presentation by class-II-transfected fibroblasts[23]. If confirmed, this would finally give a much sought function to the Ii molecule.

There are some exceptions to the rule of co-expression of class II and Ii. Konrad Reske (Mainz) observed that surface expression of class II could be induced with IFN-γ on bone-marrow-derived macrophages. Although Ii mRNA was induced in these studies, no translation was observed[24]. In the case of rat class II molecules, Reske observed that the same class II molecule can occur in two allosteric conformations which can be differentiated with the Ox6 and Ox3 antibodies which could add to the functional polymorphism of class II antigens[25].

Suppressor T cells

During the last few years, work on antigen-specific suppressor T (T_s) cells has met with increasing scepticism. However, in Münster, E. Kölsch and his group persevered with their work on T_s cells which can be induced with low doses of antigen[26,27]. These T_s cells can be cloned and express an αβ T-cell receptor. Some T_s clones are restricted by I-A and others by I-E. Whereas mouse T cells in general do not express class II antigens, the T_s clones and also the T_s cells induced in the mouse clearly synthesize I-A and I-E antigens[27,28]. Kölsch speculates that the T_s clones can present antigen to themselves via these class II molecules. However, class II expression does not seem to be a prerequisite for the suppressive activity because T_s hybridomas generated with such T_s cells did not express class II (Ref. 29).

These few examples illustrate that MHC research has developed well in Germany. They also show that MHC research is still very much alive and that many unresolved questions remain, providing an exciting challenge to immunologists and biologists.

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HLA – genes and function

Elisabeth H. Weiss[1] and Andreas Ziegler[2]

Although the cradle of the genetics and function of the human leukocyte antigen (HLA) complex did not stand in the Federal Republic of Germany, there have been many German contributions to this field. The following brief account of these activities cannot, of course, be complete but reflects our own view of a particular subject.

In parallel with a number of scientists from the UK and the USA, our own groups have collaborated on the construction of a physical map of the HLA complex (Fig. 1), by assigning individual genes to large restriction enzyme-generated DNA fragments that have been separated by pulsed field electrophoresis (or related) techniques[1]. We used mutants with induced deletions of HLA genes or even monosomy 6 for this, to minimize interpretative problems due to DNA polymorphisms. Our data show that the organization of different HLA haplotypes is apparently very similar.

Genotyping studies have been carried out by several groups to define disease-associated alleles. E. Albert and his colleagues (Immungenetisches Labor, Kinderpoliklinik, Universität München) are involved in the study of HLA class II and class III gene polymorphisms in various HLA-associated diseases, such as narcolepsy, myasthenia gravis or cortical adrenal hyperplasia[2]. They have found that linkage disequilibrium between the various alleles of the different loci from DRA to DQA2 is so strong that class II five point (locus) haplotypes can be deduced from the phenotypes in nearly all cases. C. Rittner, P. Schneider and their coworkers (Institut für Rechtsmedizin, Universität Mainz) have a longstanding interest in complement genes, some of which are part of the HLA complex. Their description of a distant, rare restriction fragment length polymorphism (RFLP) which is invariably associated with the presence of the BF*F allele[3] supports the previously observed lack of recombination between BF and the C4 genes. The disease association of HLA-B27 with spondylarthropathies was first studied at the molecular level by G. Riethmüller, E. Weiss and colleagues (Institut für Immunologie, Universität München) with cosmid clones encompassing the HLA-B27 locus[4].

Contrary to the expectation of most HLA geneticists, the first nearly complete sequences of HLA class II α and β chains were not provided by molecular biologists, but by a large group of protein biochemists headed by N. Hilschmann at the Max-Planck-Institut für Experimentelle Medizin in Göttingen. In a Herculean effort, they isolated, from hundreds of litres of culture fluid, enough HLA homozygous cells to purify class II molecules in sufficient quantity for conventional protein sequencing[5,6].

HLA antigen expression studies strongly rely on the availability of monoclonal antibodies against polymorphic or common determinants on HLA surface molecules. Such reagents have been produced by several groups, in particular those of J. Johnson (Institut für Immunologie, Universität München: especially anti-class II reagents), E. Westphal (Abteilung Immunologie, Universität Kiel: anti-class I alloreagents) and A. Ziegler (anti-class I, II (allo)reagents).

C. Müller and her colleagues (Medizinische Klinik, Universität Tübingen) have carried out a very extensive immunohistological analysis of HLA class II molecules of the major lymphoid organs and a few other cell types[7]. Their most interesting result is probably that HLA-DQ antigens are expressed in a much more restricted fashion than HLA-DR and HLA-DP molecules. Differential regulation of class II gene expression

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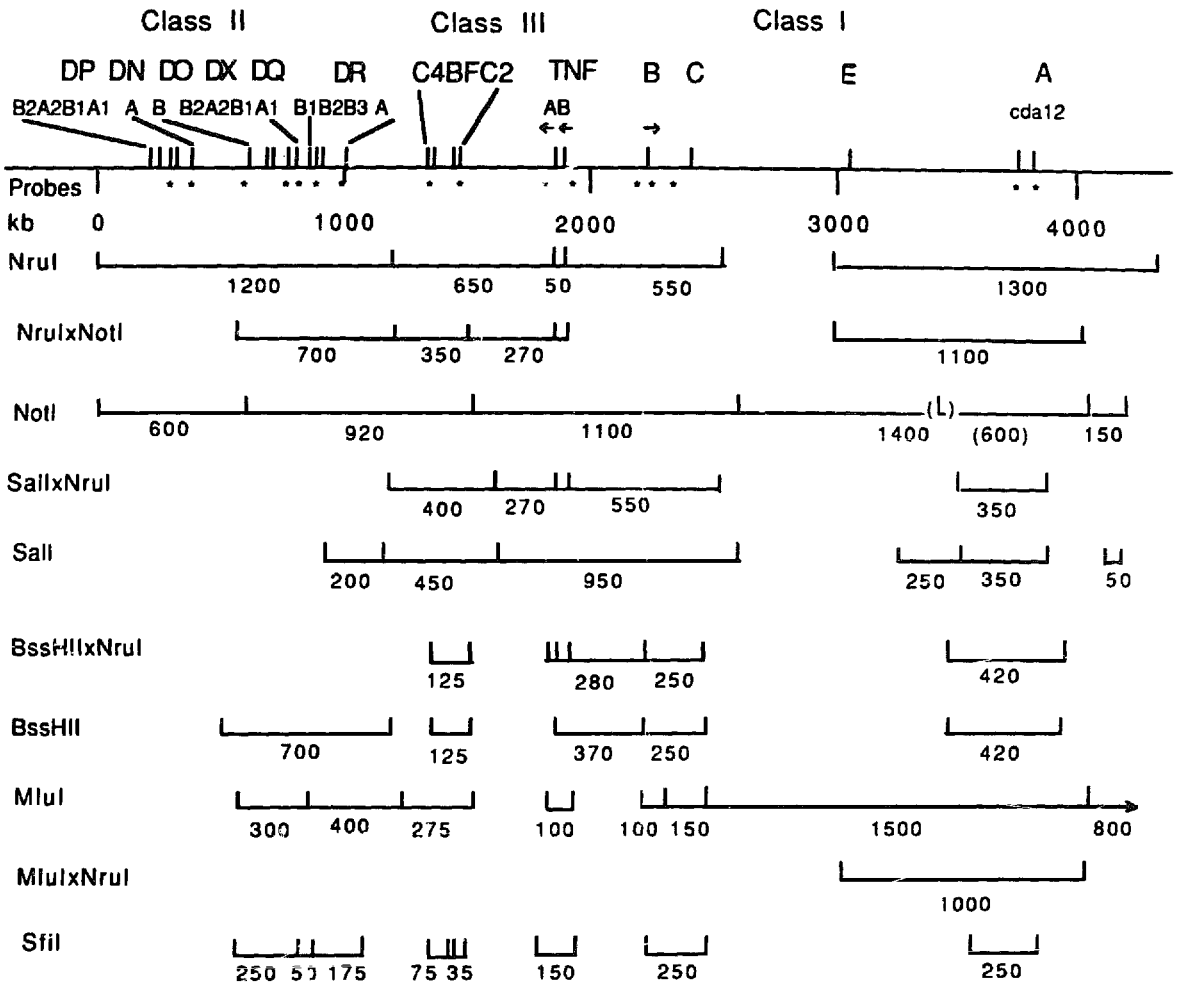

Fig. 1. *Large-scale restriction map of the HLA complex (A2, B13, Bw4, DRw6, DRw52, DQw1, DPw2 haplotype). The restriction fragments are indicated by horizontal lines and the sites by vertical bars. Genes, probes (*) and physical distances are indicated on top; the arrows show the direction of transcription. The positions of the HLA-A, E and cda 12 loci are only approximate (from Ref. 1).*

on activated T cells has been studied in the laboratory of D. Schendel (Institut für Immunologie, Universität München). Whereas DQ molecules are expressed by CD4+ cloned T cells, these antigens and the respective mRNA are not synthesized by CD8+ cells[8]. Similar results for B lymphocytes have been described by M. Hadam and his co-workers (Abteilung für Kinderchirurgie, Medizinische Hochschule, Hannover) who established HLA class-II-deficient B-cell lines from patients with HLA class II deficiency. They showed that some cloned cell lines re-express certain types of class II antigens, but never DQ molecules[9]. The controversial issue of HLA antigen expression on spermatozoa was addressed by several scientists from the University of Tübingen, who found that neither normal nor various types of pathological spermatozoa had detectable amounts of these molecules on their surfaces[10]. Finally, the HLA antigen expression by tumour cells has interested several German scientists. J. Johnson and her colleagues (München) have found that HLA-DR expression in progressing human melanoma is associated with metastatic spread and suggested that gamma-interferon may play a role in regulation of genes leading to phenotypic changes in this disease[11]. In a recent collaborative effort also involving groups in Kiel and Marburg, F. Momburg and his co-workers (Institut für Pathologie, Universität Heidelberg) have analyzed several colon carcinomas and found not only complete loss of class I antigens in several cases, but also differential loss of HLA-A or HLA-B molecules from tumour cells[12], usually of only one haplotype.

G. Hämmerling and his group (Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, Heidelberg) were the first in this country to obtain HLA class I transgenic mice. By introducing the HLA-Cw3 gene into C57BL/6 mice, they demonstrated that the Cw3 molecule has immunological functions comparable to those of the HLA-A and HLA-B antigens[13].

For several years, functional assays to clarify the role of class II gene products have been used by G. Pawelec and his colleagues (Medizinische Klinik, Universität Tübingen). They used class II antigen-specific monoclonal antibodies to block

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stimulation of T-cell clones, and recently provided evidence for the existence of class II determinants (termed 'DY') which stimulate autoreactive CD4+ T cells with suppressive activity[14].

Finally, a number of groups in West Germany have studied the association of HLA alleles with particular diseases using classical serology. H. Grosse-Wilde (Institut für Immungenetik, Universitätsklinikum, Essen) investigated the role of HLA antigens in patients with drug-induced pseudolupus[15]. They observed an increase in the frequency of HLA-DR4 and a decrease of DR3 in the patients as well as an association of acute lymphoblastic leukemia with the HLA-Cw7 antigen in a collaborative study with C. Müller (Tübingen)[16]. In view of the presence of human homologues of murine major histocompatibility complex (MHC)-linked t complex genes, the finding of C. and G. Müller-Eckhardt (Institut für Klinische Immunologie und Bluttransfusion, Universität Giessen) of male segregation distortion in narcolepsy families may be of interest[17]. Equally puzzling is the finding of G. Rittner (Institut für Humangenetik, Universität Bonn) and her colleagues who established an HLA-linked increased chromosomal breakage rate in systemic sclerosis patients[18]. Furthermore, a strong association between herpes simplex virus-induced erythema multiforme and HLA-DQw3 has been shown by R. Wank and his coworkers (Institut für Immunologie, Universität München)[19]. This appears to be the strongest association reported to date between a virus-induced disease and an HLA class II antigen.

We would finally like to mention the continuous support which basic and applied research in the HLA field has received over the recent years in West Germany. This financial support has mainly come from the Deutsche Forschungsgemeinschaft through local 'Sonderforschungsbereiche' (special research areas) as well as programmes like 'Immunogenetics of the MHC' which have provided a very useful platform for interaction for all German scientists involved in MHC research.

The research of the authors mentioned in this article has been supported by the Deutsche Forschungsgemeinschaft through SFB 120/A4, SFB 217/A1, Zi268/2–1 and the Genzentrum München.

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Complement research

M.P. Dierich[1] and D. Bitter-Suermann[2]

German immunologists have shown a traditionally strong interest in the biological activities of complement since the late nineteenth and early twentieth century – as documented by the work of H. Buchner, P. Ehrlich, E. Brand, R. Pfeiffer, F. Friedberg and H. Ritz – and this interest enjoyed a renaissance around 1960.

Rebirth of German complement research

The fathers of the present generation of complementologists are P. Klein, K. Rother, W. Vogt and H. Müller-Eberhard. Their initiative and the active support of the Deutsche Forschungsgemeinschaft, have allowed most postdoctoral researchers in the complement field to be trained at a few centres here or abroad. Besides their own substantial contribution to complement research, their main efforts were in the instruction of students.

P. Klein started his research in 1962 at the Institut für Medizinische Mikrobiologie in Mainz. In association with H.J. Wellensiek, his work at the time centered around the description of the 'classical C3 complex', leading to the identification of the components C3, C5, C6, C7, C8 and C9. Many 'second generation' complementologists, such as H.J. Wellensiek, W. Opferkuch, D. Bitter-Suermann, U. Hadding, M.P. Dierich, M. Loos, R. Burger and H.P. Heinz, now continue this work in their own groups and institutions. Their contributions, and those of their coworkers, to the field of complement research are discussed below.

K. Rother and U. Rother started working in Freiburg at the Max-Planck-Institut für Immunbiologie and continued in Heidelberg at the Institut für Immunologie und Serologie. Their main contributions were the description of a genetic C6-deficiency in rabbits, of a leuco-

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cyte mobilizing factor, generated from C3 and later identified as C3e, and studies on the role of C5 in initiating the lytic events (deviated lysis) of complement. Later, with their co-workers (C. Till, G.M. Hänsch, E.W. Rauterberg and R. Burger) their interests broadened to many different aspects of complement.

W. Vogt in Götlingen at the Max-Planck-Institut für Experimentelle Medizin was more interested in the specialized field of anaphylatoxic peptides, their enzymatic generation and their promolecules. He was one of the first to characterize C5a (porcine). His co-workers, V. Brade, the late B. Damerau and I.V. Zabern, contributed to the renown of this lab.

Finally, H. Müller-Eberhard from Scripps Clinic and Research Institute, La Jolla, now chairman of the Bernhard Nocht-Institut für Tropische Medizin, Hamburg, supported the rebirth of complement research in Germany and from the very beginning was a major contributor to it. He trained several postdoctoral fellows (such as U. Hadding, the late V. Bokisch, O. Götze and H. Horstmann) who, on returning to Germany, continued in this rapidly expanding field.

In addition, strong connections with the laboratories of M.M. Mayer (Baltimore), T. Borsos, (NIH) and H. Colten (Harvard, now at St. Louis), favoured the input of new ideas and methods, and many German postdoctorates received excellent training there.

The research environment, the scientific atmosphere, the personalities of the individuals mentioned above and their enthusiasm for complement research favoured the success of their protégés. Over the past two decades, several groups have concentrated their efforts on the biochemical characterization of the various complement components and as this issue is now largely settled, interest has shifted to the biological, clinical, molecular, genetic and pharmacological aspects. The receptors for complement fragments as links to the numerous cellular activities modulated by these peptides, are being intensiveiy studied.

Classical pathway

Two groups at the Institut für Medizinische Mikrobiologie, Johannes Gutenberg Universität of Mainz, work on the classical complement pathway. M. Loos and colleagues are studying the biosynthesis and basic mechanisms of activation of the components of the classical pathway, as well as clinical disorders of these components. Their work covers the biosynthesis of C1q by macrophages at the molecular level, (including gene expression), conformational changes occurring during activation of C1, detection of antibodies against C1q *in vivo* and analysis of complete C1q deficiency and acquired C1-inhibitor deficiencies in humans. Together with M. Loos, H.P. Heinz's group is investigating the biological role of the collagenous portion of C1q *in vitro* and *in vivo*. This work suggests a crossreactivity of the collagen-like C1q with the different types of collagen and a pathophysiological role for the C1q molecule in chronic inflammatory processes, such as rheumatoid arthritis. They are also studying the genetics of the classical pathway components C4 and C2.

Complement polymorphism

C. Rittner (Institute of Legal Medicine, Johannes Gutenberg University of Mainz) has contributed from the very beginning to the characterization of the genotypic basis of complement polymorphisms, their allotypic variations and human complement deficiencies. He is particularly interested in the human leukocyte antigen (HLA)-linked class III gene products (C2 and C4). He and P. Schneider focus on the genetic basis of C4 polymorphism and C4 deficiencies and on the biological role of C4A and C4B gene products and the susceptibility of individuals lacking them to autoimmune disorders. For more than a decade, G. Mauff from the Institute of Hygiene, Cologne, has contributed to the same field – particularly the genetic basis of C4, C2 and factor B polymorphisms.

D. Bitter-Suermann and his coworkers at the Institut für Medizinische Mikrobiologie, Medizinische Hochschule Hannover, also study genetic polymorphisms and complement deficiencies of C4, C2, C3 and C3a receptors in guinea pigs. E. Böttger in Bitter-Suermann's group proposed a theory to explain how these deficiencies lead to an impaired humoral immune response to T-cell-dependent antigens and to humoral autoimmune reactions, based on an immunoregulatory function of C3 and its fragments. This group also studies the biological activities, structural requirements and receptors for the anaphylatoxic peptides C3a and C5a. The detailed description of a test system for these peptides using guinea-pig platelets (ATP-release), which is sensitive, stimulus-specific and reproducible (S. Meuer), stimulated research on receptors for C3a and its synthetic analogues (R. Gorardy), ELISAs for the peptides in plasma with monoclonal antibodies (A. Klos), and investigations on the role of peptides in the pathogenesis of acute respiratory distress syndrome (ARDS) in a guinea pig model (T. Hoffmann and J. Köhl).

Alternative pathway

In W. Vogt's lab. at the Max-Planck-Institut für Experimentelle Medizin, Göttingen, I.V. Zabern analyzed functional and structural changes that can be induced in uncleaved C3 and C4 molecules by exposing them to amines, chaotropes or by repeated freezing and thawing. These studies contributed to the understanding of the activation of the alternative pathway by the C3b-like form of C3. Interest in the question of whether an altered C3 similar to their studies might be generated by xenobiotic agents and thus contribute to pathological complement-mediated effects *in vivo* led to the discovery of the conversion of C3 to C3b-like C3 by drugs, such as ionic iodinated contrast media, penicillins and sulfonamides. A comparison of the properties of cobra venom factor from the two species *Naja naja* and *Naja haja* revealed differences in C5 binding and usage.

V. Brade (Institut für Klinische Mikrobiologie, Universität Erlangen) has worked on the alternative complement pathway – formerly called the properdin system – since the 1970s. He characterized the role of factors B and D in the C3- and C5-activating (anaphylatoxin-forming) enzymes on zymosan. In studies with mouse and guinea pig macrophages and with liver synthesis and secretion of D, B and P in addition to C3, synthesis of single chain pro-C3 by liver cells and inactivation of C3a – resulting from cleavage of secreted

C3 – by a macrophage-derived monocarboxypeptidase was shown. His recent experiments deal with the influence of bacterial surface components on the alternative pathway activation with *Yersinia enterocolitica* as a model.

At the Bernhard Nocht Institut für Tropische Medizin, Hamburg, R. Hausmann works on the molecular mechanisms that pathogens, particularly group A streptococci and *Yersinia enterocolitica* use to evade activation of the alternative pathway.

Complement components in health and disease

O. Götze and his co-workers at the Georg-August-Universität, Göttingen, work on the evaluation of the role of complement in disease (for example in renal failure, sepsis and the adult respiratory distress syndrome) using ELISA procedures and monoclonal antibodies to plasma C3a, activated C3, C5a, factor D, Ba and Bb as well as C6, C7 and SC5b-9.

They also study the role of complement and complement peptides in cellular regulation and in the modulation of the activation/differentiation of B cells and T cells, monocytes and granulocytes in health and disease. In addition, the expression of complement proteins in blood leukocytes and other cells is evaluated by the detection of mRNA and *in-vitro* synthesis of the respective proteins. The expression of cytokines in response to complement peptides is currently being investigated using these methods. R. Buger (Robert Koch-Institut, Berlin) works on C3, mainly identifying the functionally relevant molecular sites and their interaction with other complement and membrane proteins. These *in-vitro* studies are complemented by studies in genetically C3-deficient guinea pigs.

Control factors and receptors

W. Vogt (Max-Planck-Institut für Experimentelle Medizin, Göttingen) began his complement research in the early 1960s. He recognized 'anaphylatoxin' as a cleavage peptide of a precursor plasma protein and was first to purify it, confirming Jensen's finding of a relation to C5. A cobra venom factor, (CVF) was recognized as an anaphylatoxin-releasing substance and was also purified. A heat-labile cofactor essential for the activity of CVF was discovered in serum, and later identified as factor B. The interaction of factors B, D and CVF or C3b was analyzed and it was established that factor D could be replaced by trypsin. The reason that C5 activation is restricted to surfaces and the role of C3b as a ligand for native C5 rendering it accessible to either convertase was also recognized. Recently, a non-enzymic mechanism of C5 activation was discovered – oxidation by OH radicals. This indicates a positive feedback in inflammation, since the radicals are products of complement-stimulated leukocytes. B. Damerau in Vogt's lab was the first to show that purified C3a and C5a-desArg induce platelet aggregation. The biological role of the amino-terminal region of C3a was described.

The research of M.P. Dierich, Institut für Hygiene, Universität Innsbruck, and of his co-workers (T.F. Schulz, C. Larcher, H.P. Huemer, W. Schwaeble, J. Möst and B. Sölder) centers around complement receptors (CR1, CR2, CR3) and their biological functions, their structure and physiological regulation. They are investigating the influence of the human immunodeficiency virus (HIV-1) and of the human T lymphotropic virus HTLV-1 on CR2 and CR3. A receptor for iC3b was discovered on *Candida albicans* and its biochemistry and molecular genetics were analyzed, as was the 'receptor' for C3b on *Herpes simplex* virus. The regulator protein factor H was analyzed; three messenger RNAs were discovered and regulation of transcription in different cells is now being investigated.

C3 activation products and T-cell interaction

U. Hadding, B. Faßbender and W. Däubener at the Institut für Medizinische Mikrobiologie und Virologie, Universität Düsseldorf, study the interaction of C3 split products, particularly C3b and C3d, with macrophages and T cells. They showed that C3b as well as C3a could trigger the metabolism of arachidonic acid, resulting in a decrease in the expression of IA antigens after C3b treatment of guinea pig macrophages that leads finally to a reduction of T-cell proliferation in antigen driven systems. They also found evidence that C3d is responsible for further inhibiting T-cell proliferation, by interfering not only with interleukin 2 (IL-2) but also with IL-3 and IL-4 function *in vitro*. They have postulated the presence of a CR2-like receptor on T cells.

C6–C9

Finally, within the complement cascade the components C6–C9, which form the lytic membrane attack complex C5b–C9, are the subject of research by three groups. At the Institut für Immunologie und Serologie, Universität Heidelberg, K. Rother and his co-workers (M. Kirschfink, G. Zitow, G.M. Hänsch, E.W. Rauterberg and U. Rother) examine the mediators of immune inflammatory reactions and the development of better diagnostic tools to assess complement activation *in vivo*. To focus on two topics, G.M. Hänsch described a membrane (C8-binding) protein that inhibits C-mediated lysis in homologous systems. In addition, she investigated the involvement of C5b–9 in the progress of acute and chronic inflammation by analyzing the response of cultured glomerular or synovial cells to C5b–9. Eicosanoids, IL-1 and other mediators were released. E.W. Rauterberg analyses target-cell destruction by lytic molecules *in vitro* and *in vivo*. Monoclonal antibodies to C9 blocked different functions of the protein. Immunohistology revealed MAC (C5b–9) deposits in a variety of autoimmune diseases. MAC pathogenicity was also established in experimental animals.

At the Institut für Medizinische Mikrobiologie, Universität Gießen, S. Bhakdi's main efforts are directed at the molecular and functional analysis of interactions between the terminal components C5–C9, investigations on the modes of activation and control of the terminal sequence and studies on the biological functions of terminal complement complexes. Finally, Bhakdij and his co-workers are studying the bactericidal action of complement and exploring mechanisms that augment complement-killing of serum-resistant organisms.

Without going into too much detail of the 'complement landscape' in Germany, we hope that we have shown that complement research is an active and dynamic branch of immunology in our country.

Lymphokines

P.H. Krammer[1], H. Kirchner[2] and A. Schimpl[3]

Since the original discovery by A. Schimpl and E. Wecker (Würzburg) that soluble factors (T-cell replacing factors) replaced T cells *in vitro* in T-cell-dependent, B-cell anti-sheep red blood cell responses[1], the investigation of lymphokines (or more correctly cytokines) has made considerable progress in the Federal Republic of Germany. With the advent of T-cell cloning, refined cellular techniques, molecular biology, sophisticated biochemistry and monoclonal antibodies, the obscure mixture of factors in T-cell supernatants has turned into the reality of purified molecules. Today, lymphokines are a major tool in basic and applied immunological research and have begun to establish their place in clinical medicine. Thus, their role in growth, activation and differentiation of lymphoid and non-lymphoid cells is almost as diverse as the number of investigators and clinicians using them daily in the laboratory and hospital. In this sense, many more laboratories in Germany than those directly cited here play an active part in lymphokine research on a broader scale. This overview of activities in this field of research in the Federal Republic of Germany mainly includes those whose primary research topic is 'lymphokines'. Here we describe some of these research activities, following the current concept of activation of T cells – which are the main producers of lymphokines – and also discuss the effect of lymphokines on other cells.

Helper T cells are activated by direct contact with antigen-presenting cells and interleukin 1 (IL-1) and possibly other cytokines, including receptor-inducing factors (RIFs) secreted by these cells. IL-1 binds to its receptor on helper T cells, is probably internalized, and initiates a number of effects that lead to T-cell growth and activation. Epitopes that are important for IL-1 activity as determined by monoclonal antibodies, the receptor for IL-1 and the process of IL-1 internalization and its intracellular effects are investigated by the groups of H-D. Flad[2] (Borstel), K. Resch[3] (Hannover) and P. Krammer[4] (Heidelberg), respectively. RIFs were originally described by T. Diamantstein[5] (Berlin) and seem to be mostly macrophage products; these are now being investigated by H. Wagner and co-workers in Ulm[6].

After these initial events, activated helper T cells secrete a number of lymphokines. These include IL-2 and IL-4, which after binding to their respective receptors have autocrine or paracrine growth factor activity on T cells. These lymphokines and their receptors are the focus of interest of many groups: Wagner and co-workers (Ulm) are investigating which T cells on a clonal level secrete these lymphokines[7]; Krammer and colleagues (Heidelberg) and Schimpl and colleagues (Würzburg)[8], are studying the regulation of IL-2 (Schimpl) and IL-4 (Krammer[9]) gene expression and the relevant 5′ DNA sequences (Serfling, Würzburg[10]). The role of IL-4 and its receptor in autocrine growth stimulation of normal and malignant T cells is also being studied by Krammer (Heidelberg)[11]. Sebald (Würzburg)[12] has determined the epitopes on IL-2 that are important for IL-2 receptor binding, while T. Diamantstein in Berlin has greatly contributed to the characterization of the IL-2 receptor in mice and rats using monoclonal antibodies[13,14]. He has shown that such antibodies can be used to abrogate T-cell activity and growth in autoimmune diseases (type 1 diabetes in the rat)[15] and graft rejection[16]. E. Rüde and A. Reske-Kunz and their associates in Mainz have investigated the signals required for the kinetics of IL-2 receptor expression in T-cell clones after activation and the possible physiological role of soluble IL-2 receptors[17].

Activated helper T cells secrete lymphokines which have pleiotropic effects on the secreting T cells, T cells of the same or different subsets, B cells, macrophages and cells outside the immune system. The T-cell-tropic lymphokines include T-cell cytotoxicity inducing factor 1 (TCF-1), which induces growth and maturation of precursors of cytotoxic T cells (W. Falk, Männel, and W. Dröge, Heidelberg[18]). Lymphokines with activity on B cells include T-cell replacing factor (TRF) and IL-4. Late TRF activity as initially described was thought to be mediated by one factor but has proved to involve various factors (for example, IL-4, IL-5) depending on the type of B cell studied and the degree of antigen receptor engagement (A. Schimpl and E. Wecker, Würzburg). The work on IL-4 as a lymphokine that induces isotype switching of lipopolysaccharide prestimulated murine B cells towards IgG1 and IgE, was started by Vitetta (Dallas), P. Krammer (Heidelberg)[19], Coffman (Palo Alto)[20] and their groups and is also investigated by K. Rajewski's group (Cologne), by A. Radbruch and Müller and their associates. Their results, using molecular biological techniques, suggest that IL-4, as previously suspected from immunoglobulin isotype tests with B cells in limiting dilution, actually induces immunoglobulin isotype switching rather than expanding B-cell clones which have already switched to a particular immunoglobulin isotype[21]. T-cell derived lymphokines also stimulate macrophages and lead to their activation to different functions. Signals important for macrophage activation are investigated by the groups of D. Gemsa (Marburg)[22], H. Kirchner (Lübeck)[23], P. Krammer (Heidelberg)[24], M. Lohmann-Matthes (Hannover)[25] and Röllinghoff (Erlangen)[26]. Since gamma-interferon (IFN-γ) has emerged as the first macrophage activating factor (MAF) acting in conjunction with lipopolysaccharide, a number of different MAFs has been described which activate macrophages either alone or in combination with IFN-γ. Thus, IL-4 and colony-stimulating factors (CSFs) can have MAF activities. In addition, P. Krammer and D. Gemsa showed that IFN-γ in combination with lymphotoxin and tumor necrosis factor (TNF) activate macrophages.

Macrophages activated by T-cell derived lymphokines or directly by tumor cells (D. Männel, Heidelberg)[27] secrete potent cytokines such as

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CSFs, IFN-α and TNF. These cytokines are the focus of interest in many laboratories of academic institutions and the biotechnology industry. H. Kirchner's group investigated the importance of endogenous interferons in viral diseases and was the first to show that TNF also has antiviral activity[28]. D. Männel, V. Lehmann, and W. Dröge (Heidelberg) and K. Pfizenmeier, Krönke and P. Scheurich (Göttingen) and their associates are investigating induction of TNF secretion from macrophages, autostimulatory activity of TNF, TNF resistance, TNF receptors, and signals induced after TNF binding and other aspects of this potent mediator[29–31]. Krammer's group has developed a neutralizing monoclonal antibody against murine lymphotoxin and TNF which allows these cytokines to be tested in *in-vivo* murine models[32].

Lymphokines are expected to have a considerable impact on the understanding of the pathogenesis of diseases and on clinical therapy. Continuous stimulation of growth by autocrine growth factors of malignant B cells, for example, may be an important feature of this malignancy (K-M. Debatin and P. Krammer, Heidelberg)[33]. The hypothesis of autocrine growth stimulation may also be fruitfully extended to other malignant diseases in which autocrine loops are found. In clinical therapy in many centers, several lymphokines have entered a trial phase and some have moved from trial to routine. Various clinical investigators including Mertelsmann (Mainz)[34], Welte (Hannover)[35], Porzsolt (Ulm)[36] and von Wussow (Hannover)[37] have shown that the transition from experimental knowledge to clinical usefulness can be quick. Reconstitution of patients with depressed bone marrow function can be induced by injection of CSFs, particularly granulocyte–macrophage-CSF. This therapy is now frequently used and has proved beneficial to many patients. It has decreased infections and shortened the hospital stay of patients, such as those with bone marrow transplants. Furthermore, interferon has become instrumental in the therapy of laryngeal papilloma, hairy cell leukemia and chronic myelogenous leukemia and is also used in Kaposi Sarcoma treatment. In addition, local co-injection of IL-2 with a hepatitis B vaccine led to anti-hepatitis B virus antibodies in otherwise immunodeficient patients (S. Meuer, Heidelberg)[38]. These examples, therefore, show that lymphokines will play an ever increasing role in research and clinical medicine.

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Cellular signalling in T lymphocytes

Stefan Meuer[1] and Klaus Resch[2]

Activation of resting T lymphocytes to proliferation and terminal differentiation is a multistep process involving synthesis of interleukin 2 (IL-2), the receptors for it, and the interaction of both. Effective synthesis of IL-2 requires IL-1, the receptors for which must also be expressed. The biology of the cytokines and their receptors is dealt with in a separate contribution: here only the signalling of those receptors that initiate this chain of events will be discussed: the T-cell antigen receptor (TCR) composed of the Ti–CD3 complex and the CD2 glycoprotein.

TCR signal transduction

Owing to the diversity of the TCR and the complex nature of the appropriate antigen, early studies of T-cell triggering relied exclusively on models using mitogens such as plant lectins. Since the introduction of activating monoclonal antibodies (mAbs), the signalling of the TCR can be studied more directly. The most commonly used mitogens, phytohemagglutinin (PHA) or concanavalin A (ConA), activate T cells through components of the Ti–CD3 complex and thus can be used to probe this receptor in species where specific antibodies are lacking (for example, see Ref. 1).

The first well defined cellular signalling system consists of cyclic nucleotides. Although many reports have been published on their role in lymphocyte activation, it now appears that neither cAMP nor cGMP are involved as second messengers in activated T lymphocytes, but they may for example exert regulatory functions in response to hormones[2].

Several receptors elicit cellular responses by breakdown of phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and diglyceride, by activating a PIP_2-specific phospholipase C ('PI response'). IP_3 raises cytosolic free Ca^{2+} by releasing it from internal stores, and after phosphorylation to IP_4, by gating Ca^{2+} channels of the plasma membrane. Diglycerides, together with Ca^{2+}, translocate the cytosolic protein kinase C (PKC) to the plasma membrane, and thereby activate the enzyme. The activated PKC phosphorylates proteins involved in specific cell responses.

Binding of activating ligands to the Ti–CD3 complex induces a rapid PI response. Although the majority of phospholipase C in rabbit thymocytes is found in the cytoplasm, this key enzyme can be clearly demonstrated in highly purified plasma membranes, which also contain the substrate PIP_2; this is consistent with their role in signal generation[3]. Inhibition of the PI response by cholera toxin (which specifically ADP ribosylates GTP binding proteins, linking receptors to effectors such as adenylate cyclase (G-proteins)) had suggested that the TCR is coupled to phospholipase C in T cells by a G-protein. In a human cytotoxic T-cell clone which was permeabilized by α toxin, H. Schrezenmeier and B. Fleischer (Ulm) showed that non-hydrolyzed GTP analogues induced granule exocytosis. This was strongly dependent on PKC, suggesting that the G-protein involved in this cell activation is localized in signalling steps proximal to phospholipase C (Ref. 4). More directly, H. Sommermeyer and co-workers (Hannover) showed that the same GTP analogues specifically activated the phospholipase C in isolated plasma membrane from rabbit thymocytes[5].

Evidence that this G-protein is linked to the TCR – and thus couples it to phospholipase C – again comes from experiments with permeabilized T-cell clones, in which modulation of the Ti–CD3 complex by mAbs led to a state of nonresponsiveness to stimulation by GTP analogues[6]; however responses by direct PKC activators, such as TPA, could still be elicited[7]. This correlates with findings that treatment of the human Jurkat T lymphoma cell line with cholera holotoxin led to a rapid and specific loss of the Ti–CD3 complex, whereas other membrane proteins were not affected (H. Sommermeyer *et al.*, submitted). The nature of this specific G-protein is not yet known, but in plasma membranes of human lymphocytes at least G_s, G_{i1}, G_{i3} and G_z are present (H. Sommermeyer and K. Resch, unpublished).

The activation of phospholipase C constitutes signal branching giving rise to more than one second messenger, an elevation of cytosolic free Ca^{2+} and formation of diglycerides, which activate PKC. Inhibition of cellular responses with (unfortunately rather nonselective) PKC inhibitors suggested that this enzyme is critical in T-cell activation.

That both Ca^{2+} and PKC activation are required and sufficient for proliferation of T lymphocytes was shown elegantly in experiments using PKC-activating phorbol esters such as TPA in conjunction with Ca^{2+} ionophores. B. Manger (Erlangen) while working in Art Weiss' group, contributed significantly to this two signal model for T-cell proliferation[1,8]. This approach established that the receptor-mediated events could be successfully bypassed experimentally and, on the other hand, strongly supported the essential role of phospholipase C-dependent generation of diglycerides and IP_3 in receptor-mediated physiological signalling. In contrast to the phorbol esters, however, the physiological diglycerides are short lived, and, hence PKC activation is also transient (less than 30 min) and reversible. When membrane permeable diglycerides were used, instead of phorbol esters, only IL-2 receptor expression was observed, while IL-2 synthesis and subsequent proliferation were absent (see also Refs 1 and 8). The latter could be induced by repeated addition of diglyceride, suggesting (together with other evidence[1,8]) that short-term activation of PKC suffices for IL-2 receptor expression, but sustained activation is required for transcription of IL-2 message. M. Szamel, Hannover, provided experimental evidence for a mechanism operating physiologically. In human peripheral lymphocytes stimulated

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with Ca^{2+} ionophores and short-lived diglycerides, incorporation of *cis*-polyunsaturated fatty acids such as linoleic acid or arachidonic acid into membrane phospholipids led to a sustained activation and translocation of PKC. Those stimulated cells produced large amounts of IL-2 and subsequently proliferated.

When the lymphocytes were stimulated with mAbs against the Ti–CD3 complex, PKC activation proved to be biphasic. A transient first phase of activation, which until then was the only one observed, could be attributed to the PI response, and was followed by a second, sustained PKC activation, which became apparent only after one hour of stimulation – putatively due to a substitution of membrane phospholipids with polyunsaturated fatty acids (M. Szamel *et al.*, submitted).

Changes in the phospholipid fatty acid metabolism have previously been analyzed extensively in mitogen-stimulated lymphocytes. In the plasma membrane the deacylation–reacylation cycle was activated involving activation of phospholipase A_2 and lysophosphatid acyltransferase, through which saturated fatty acids were substituted by polyunsaturated ones. Increase in the content of polyunsaturated phospholipid fatty acids thus became measurable after about one hour of stimulation[9,10]. Recently, human lymphocytes, this time stimulated with antibodies to the Ti–CD3 complex, showed similar changes (M. Szamel *et al.*, submitted). Interestingly, in mitogen-activated rabbit lymphocytes, cyclosporin A, which does not interfere with the PI response, completely prevented the activation of the phospholipid fatty acid metabolism[11]. This may well explain why cyclosporin A prevents IL-2 synthesis, but does not inhibit the synthesis and expression of IL-2 receptors.

It is thus proposed that this 'phospholipid polyunsaturation' (PP) response constitutes the long sought for second signal system, which, in addition to the PI response, is coupled to the TCR, and required for initiating IL-2 synthesis and proliferation.

T-cell activation via CD2

Besides direct triggering through the Ti–CD3 T-cell antigen–receptor complex[12], T cells can be induced to proliferate and to differentiate functionally following binding of mAbs to epitopes of the 50 kDa CD2 glycoprotein[13]. This molecule is expressed by the vast majority of T lineage cells. It was initially suggested that this mode of T-cell triggering represents an alternative pathway of T-cell activation. Whether, in fact, this CD2-related mechanism allows T cells to be activated in the absence of antigen receptor triggering, that is in a non-specific fashion *in vivo*, is not yet known. Several authors have suggested an interdependency between CD3 and CD2, based on experiments with T-cell receptor deficient mutants of human tumor cell lines showing that analogous intracellular events occur following triggering of Ti–CD3 or CD2.

Other evidence, however, supports the independence of CD2-mediated T-cell activation from a functional T-cell antigen–receptor complex. For example, human T lymphocytes have been cloned and expanded which express Ti–CD3 but not CD2 (Refs 14, 15). Conversely, a population of TCR^-CD2^+ cells has been identified and studied in detail[16,17]. The latter population is clearly susceptible to activation via CD2. Moreover, Fc receptors (defined by anti-CD16 mAbs) contribute to triggering natural killer lymphocytes[18]. In addition, circulating T lymphocytes in a patient with ataxia teleangiectasia[19] are unresponsive to CD2-dependent stimuli, despite expression of a functional Ti–CD3 complex, the CD2 molecule, and normal *in-vitro* responses following activation via Ti–CD3.

In certain physiological and pathological conditions, these two pathways apparently segregate[20]. Moreover, despite normal surface expression of the two receptor systems, human intestinal lymphocytes barely respond to CD3 activation and, at the same time, are hyperreactive to CD2 triggering. The finding that these mucosal T cells constitutively express low levels of PKC supports recent suggestions that these two pathways of T-cell activation identified to date are apparently differentially regulated at the second messenger level (Pirzer *et al.*, submitted).

A most convincing piece of evidence in favour of a TCR-independent role of CD2-mediated T-cell activation was provided by analysing functional consequences of binding of CD2 to its natural ligands, LFA-3 and/or T11TS (Refs 21, 22). Both ligands, either in purified or in membrane bound form, produce a strong triggering signal to cells, which is selective for alternative pathway activation. These ligands interact with CD2 via two sites against which individual mAbs have been produced (termed anti-$T11_{1A}$ and anti-$T11_{1B}$). $T11_{1A}$ is apparently the triggering site for LFA-3/T11TS whereas, in contrast, the $T11_{1B}$ site is a binding domain for the physiological ligands (LFA-3, T11TS) (Meuer *et al.*, 1989). Reagents that selectively block the signal provided through CD2–LFA-3 have allowed the role of the CD2-mediated pathway of T-cell activation in physiological immune responses (for example to soluble antigens or alloantigens) to be studied. It was found that a considerable proportion of immune responses is apparently mediated through CD2. Given these results, it seems hardly surprising that some types of immunodeficiency are related to a failure to activate human T cells via CD2.

The existence of lymphocytes expressing both Ti–CD3 and CD2, which, however, cannot be triggered through CD2 (see above) suggest that additional molecules are required or involved in T-cell activation through the alternative pathway. This notion is formally supported by gene transfer experiments showing that transfection of CD2 genes in non-lymphoid cells is not sufficient for mediating signals such as calcium mobilization through this molecule. Such findings have prompted experiments to identify additional cell surface molecules that may be associated with CD2 in T-cell triggering. To this end, mAbs were produced and screened for their capacity to supplement a submitogenic signal mediated by antibodies directed at CD2 *in vitro*. Such antibodies were recently found and shown to be reactive with molecules of the CD45 (leucocyte common antigen) family. Interestingly, cooperation between CD2 and CD45 is apparently restricted to the high molecular weight species of CD45, namely the subunits defined by monoclonal antibody 2H4 (Schraven *et al.*, 1989).

Only a subpopulation of the CD45$^+$ cells (CD45RA$^+$) responds to anti-CD2 and anti-CD45 monoclonal antibodies whereas the reciprocal population (CD45RO$^+$) defined by monoclonal antibody UCHL-1 (CD45RO) does not. Moreover, mAb 2H4 (to CD45RA) itself is comitogenic with CD2 whereas mAb UCHL-1 is not. More important, while amplifying CD2-mediated immune responses, the same monoclonal antibodies do not affect T-cell triggering through Ti–CD3. Thus, besides LFA-3–T11TS, activation of CD45 apparently mediates an additional signal for T-cell triggering through the alternative pathway. A physical association between CD2 and CD45 has been recently shown by co-precipitation of these molecules following their cross-linking on the T-cell surface (Schraven *et al.*, submitted).

Thus the alternative pathway of T-cell activation, besides serving as an amplification mechanism in antigen-driven immune responses, may provide a means of rapid recruitment of local T cells before sufficient antigen-specific cells have been generated. If this is the case, then one would expect to find more clinical immunodeficiencies associated with failure of CD2-mediated T-cell activation. Conversely, hyperreactivity of the immune system could be attacked at the LFA-3–CD2 interaction site or at the level of CD45 molecules. Finally, CD2-mediated immune responses may play a pivotal role in local mucosal immunity.

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T-cell receptors and cellular interactions

Hermann Wagner[1] and Klaus Eichmann[2]

The functions of T lymphocytes depend on their interactions with other cells. For example, cytotoxic T lymphocytes (CTL) lyse target cells expressing the antigen used for their activation, and helper T cells induce the proliferation of antibody-producing B lymphocytes. To trigger antigen reactive T cells, cellular interactions with antigen-presenting cells (APC) are required. The function of clonally distributed T-cell receptors ($\alpha\beta$ or $\gamma\delta$ heterodimers) is to convey antigen specificity. Moreover, the function of genes encoded for by the major histocompatibility complex (MHC) is to focus antigen reactive T cells to the cell surface of other cells. MHC glycoproteins achieve this by using two apparently unrelated strategies. First, irrespective of whether antigen processing leads to MHC class I or class II presentation, stretches of specific amino acid sequences (peptides) derived by degradation from complete protein antigens bind to MHC products and are displayed on the cell surface. Second, monomorphic interactions of MHC gene products with other members of the immunoglobulin superfamily (CD4–MHC class II products, CD8–MHC class I products) increase the antigen-specific activation, presumably by providing additional positive signals. In this scenario, engagement of the T-cell receptor (TCR) represents the prime signal for T-cell activation, while it is the function of MHC gene products to 'display' processed antigen such that crosslinking – via ligand–receptor interaction – can occur. Accordingly, the functional separation into two classes of MHC recognition responses – CD4 T cells recognizing antigen bound to MHC class II molecules, while CD8 T cells recognize antigen bound to MHC class I molecules – reflects the use of an amplification device operating secondarily to TCR crosslinking.

After triggering via TCR crosslinking, antigen-specific T cells express receptors for hormone-like glycopro-

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Immunology Today, Vol. 10, No. 8, 1989 Supplement

teins produced by a variety of activated cells of the hemopoietic lineage. For example, various interleukins serve as communication signals between lymphocytes and control their clonal growth and maturation into effector cells. Although the effect of these lymphokines appears to be pleiotropic and very much dependent on the nature of the target cells, interleukin 2 (IL-2) primarily promotes T-cell growth, while IL-4 preferentially causes growth and maturation.

Several groups working in West Germany have contributed significantly to this conceptual framework. K. Eichmann and F. Emmerich (Freiburg) showed that it is the concomitant crosslinking of TCR and CD8/CD4 molecules which triggers resting, peripheral T cells to respond to the growth promoting effect of IL-2 (Refs 1, 2). On the basis of more recent data they concluded that it is the spontaneous association of CD4/CD8 molecules with crosslinked TCR which triggers T-cell activation. Interestingly, crosslinking of CD8 molecules to TCRs in intrathymically developing T cells causes immature $CD4^+CD8^+$ T cells to mature into single positive, mature T cells, suggesting a role for the CD4/CD8 structures in thymic repertoire selection. H.U. Weltzien and associates from the same group have used large panels of H-2^b-(trinitrophenol) TNP specific CTL clones to perform functional and structural analyses of their antigen receptors[3]. Accordingly, *in-vitro* cultivated CTL lines quickly select for clones using characteristic TCR types (V_α10 and J_β2.6). These results suggest the existence of immunodominant TNP determinants (TNP conjugated peptides?) presented by stimulator cells. J.T. Epplen (formerly working in Freiburg and now with H. Weckerle in Munich) has shifted his main interest from the analysis of T-cell receptor chain usages of H-2^b-TNP specific CTL clones to that of myelin basic protein (MBP)-specific T cells, and to autoreactive human T cells found in human rheumatic synovia. This group analyses the T-cell receptor α and β chains of T cells involved in the elicitation and suppression of induced antigen specific autoimmunity in animal models[4].

K. Heeg and H. Wagner (Ulm) are studying the signal requirements for the primary activation of murine CD8 T cells, and peripheral mechanisms leading to their specific unresponsiveness (tolerance). Of particular interest is the analysis and the interconnection of the IL-2- and IL-4-mediated growth pathways, and the role of IL-6 in inducing cell cycle progression. Recently, it has been found that IL-4 and IL-2 act synergistically to convey resistance to the immunosuppressive effect of cyclosporin A to clonally developing CD8 T cells[5,6]. In human fetal liver and placenta, D. Kabelitz (now in Heidelberg) has recently identified $CD2^-CD3^+$ γδ TCR bearing T cells[7], whereas $CD2^-CD3^+$ T-cell clones established from peripheral blood expressed the conventional αβ TCR. Functional studies indicated that $CD2^-CD3^+$ T-cell clones are normally responsive to signalling via CD3 or the TCR as assayed by IL-2 production, proliferation and induction of cytolytic activity. B. Fleischer (now in Mainz) uses cloned human T cells as a probe to study their signal requirements for activation. While identifying a regulatory function of CD8 and CD4 molecules, he also described an alternative, but TCR-linked pathway of T-cell activation via Tp103. The interest this received provoked the recent work of his group on the T-cell activation properties of enterotoxins from *Staphylococcus aureus* (SE). They found that SE binds to monomorphic MHC class II gene products of APCs and simultaneously triggers a variety of cloned human T cells expressing either the CD4 or CD8 phenotype[8]. This mode of T-cell activation also occurs with *Mycoplasma arthritidis* mitogens[9].

In Mainz, the groups of A.B. Reske-Kunz and E. Rüde and their collaborators are involved in studies to define the signal requirements for T-cell activation. Using an MHC class II synthesizing T-cell clone as APC, they showed that presentation of nominal antigens triggered lymphokine production in responding T cells, yet failed to entail IL-2 receptor upregulation with concomitant T-cell proliferation[10]. The as yet unidentified lymphokine(s) responsible for this effect are currently being characterized. In addition, Reske-Kunz's group have successfully transfected insulin reactive $CD4^-$ T cells with gene segments coding for CD4 to show that the transfectants exhibited not only increased antigen reactivity to insulin, but also gained reactivity to an unrelated antigen[11]. They also recently defined a $CD8^+$ T-cell clone which apparently recognizes the protein ovalbumin in a H-2^b restricted fashion, in the absence of externally added accessory cells[12]. Does this mean that clonally developing T cells can function as specialized APCs able to internalize antigen, and to process and present it together with MHC class I antigens?

T. Diamantstein (Berlin) studies the physiology of the receptor for IL-2 (IL-2R) and the possibility of inducing specific immunosuppression *in vivo* by inactivating lymphocytes using anti-IL-2R monoclonal antibodies. In several animal systems including models for graft-versus-host (GVH) reactions, autoimmune thyroiditis and autoimmune diabetes in the rat, he and his associates showed that a combination of anti-IL-2R monoclonal antibodies plus cyclosporin A effectively prevented the development of signs of disease. Interestingly, this protocol allowed detection of suppressor cells[13,14].

Although there is clear and compelling evidence that T cells can function as suppressor cells, very few groups have succeeded in defining suppressor T cells at the clonal level. Over the last few years E. Kölsch (Münster) and his group have cloned antigen-specific suppressor T cells (T_S) and analyzed their functional abilities both *in vitro* and *in vivo*. These T_S-cell clones show exquisite antigen specificity and bear 'classical' TCR αβ structures[15]. As such, these data represent a stronghold denying the currently fashionable criticism of the existence of suppressor T cells.

Experimental evidence obtained over the last few years led M. Zöller (Heidelberg) to suggest that it is the idiotypic–anti-idiotypic interconnection of cellular components which maintains the immune system in a steady state. Predictions of this concept are that self-tolerance cannot be due exclusively to clonal deletion of autoreactive clones, and that immunological reactions towards external stimuli require the establishment of a new steady state rather than activation of single populations. With a model system of tolerance induced by free hapten, M. Zöller has accumulated experimental evidence supporting this concept[16].

H.G. Thiele and his associates use the rat T-cell marker RT6 to study the ontogeny of T cells in the rat[17]. Unlike the mouse, T cells of adult rats lose Thy-1 expression, but become RT6-positive. Recently, the use of an RT6.2-specific cDNA clone showed that the inability of diabetes-prone BB rats to generate RT6+ T cells is due to a maturational block rather than to a defect of the corresponding gene. In addition, A. Hamann of this group studies the role of lymphocyte surface molecules (for example LFA-1) in lymphocyte migration and homing mechanisms[18].

In conclusion, we have briefly mentioned the work of a number of German scientists researching T-cell receptors and cellular interactions. Many questions remain unanswered but there is confidence that the problems in collating data from different model systems are lessening.

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Macrophages and accessory cells of the immune system

Clemens Sorg[1] and Marie-Louise Lohmann-Matthes[2]

In the late nineteenth and early twentieth century, German scientists – particularly anatomists and pathologists – were most prolific in investigating cellular anatomy and pathology of vertebrate organisms. Not only did they describe a variety of cells such as the Langerhans cells of the skin (1868), the Kupffer cells of the liver (1899) and the blood monocytes (Paul Ehrlich, 1881) but they also described in remarkable detail the kinetics of the inflammatory processes (J. von Cohnheim, 1873). The observations that phagocytic cells are the most prominent cell types in host defense reactions induced Aschoff in 1924 to include all cell types that shared the ability to phagocytose and store into the so-called reticuloendothelial system (RES).

The term 'accessory' cell has only recently been introduced to describe all cells that are capable of co-operating in the induction and regulation of cellular and humoral immune responses. Current literature suggests that in addition to macrophages and dendritic cells, endothelial cells may also function as accessory cells; that is, most of the cell types of the RES also seem to share this property. Metchnikoff, Ehrlich and later Aschoff entertained the notion that phagocytic cells were intimately involved in immune reactions.

More recently at the Pathology Institute at the University of Kiel the study of the origin of the blood monocyte has been resumed. Applying enzyme cytochemistry, Leder (1967) showed that blood monocytes and granulocytes share a common differentiation pathway within the bone marrow. This observation was substantiated by Parwaresch and Radzun at the same institute, by analyzing antigen expression on monocytes/macrophages using monoclonal antibodies (mAbs)[1]. With mAbs, it is possible to discriminate the phagocytosing compartment of the monocyte/macrophage system from that of immune accessory cells[2]. The latter are represented by the dendritic reticulum cells (follicular dendritic cells) as the accessory cells for humoral immune responses[3] and the interdigitating reticulum cells, Langerhans cells, indeterminate dendritic cells and veiled cells as the accessory cells for cellular immune responses. Before this, Lennert had already distinguished nonphagocytosing reticulum cells respresented by dendritic reticulum cells of lymph node follicles (follicular dendritic cells), interdigitating reticulum cells of T zones, and fibroblastic reticulum cells from phagocytosing reticulum cells (also called histiocytic reticulum cells)[4].

The Institute of Experimental Dermatology (Münster) is entirely devoted to macrophage biology. C. Sorg and his collaborators work on the role of macrophages in inflammation and focus on the question of how circulating blood monocytes find their way to the inflammation site and differentiate into macrophages. Their work includes projects on monocyte–endothelial interaction and the phenotypic dynamics of macrophages in the course of various inflammatory

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processes. In particular, the role of the macrophage migration inhibitory factor (MIF) in the differentiation of monocytes to macrophages is studied. As a spin off, this work has led to the sequencing and cloning of the cystic fibrosis antigen, which was identified as a product of myelomonocytic cells and seems to interact with MIF in a way hitherto not fully understood[5]. Furthermore, a novel angiogenic factor produced by a subset of inflammatory macrophages has recently been described[6]. With a panel of mAbs to human tissue macrophages (developed at this institute) new insights have been gained into the function of macrophages in inflamed tissues. For example, E. Bröcker (Münster)[7] showed the association of certain macrophage phenotypes with a poor prognosis in human malignant melanoma; this was confirmed by G. Heidl (Münster) on gastric carcinoma.

Phospholipid metabolism

Another center of macrophage biology started in Germany in the 1960s at the Max-Planck-Institut for Immunbiology (Freiburg), under the direction of Herbert Fischer. Together with P.G. Munder (Freiburg) he worked on the phospholipid metabolism of macrophages and with M.L. Lohmann-Matthes described lymphokine-activated macrophages as cytotoxic effector cells.

Munder and co-workers (Freiburg) studied the interaction of adjuvants and macrophages, and found that immunological adjuvants cause a drastic change in the composition of phospholipids in the cellular membrane of phagocytes. An increase of the potentially cytotoxic lysophosphatidylcholine was observed. Slowly metabolizable analogs were synthesized and were in turn found to activate normal resident macrophages, as measured by a highly increased phagocytic and anti-tumor cytotoxic capacity.

E. Ferber and his group described phospholipase A2 as a key enzyme involved in the modulation of many macrophage functions[8]. They showed that macrophage phospholipase A2 selectively releases arachidonic acid from membrane phospholipids and thus controls the generation of prostaglandins and leukotrienes. This pathway is activated by a directed translocation of inactive cytosolic phospholipase A2 to cellular membranes.

D. Gemsa and his group (Marburg) were among the first to explore the biological role of arachidonic acid metabolism in macrophages. They established macrophages as a primary source of prostaglandins and in particular of prostaglandin E_2 (PGE_2) – a potent mediator of inflammation and immunosuppression[9]. They also identified C3B and C5B/9 as potent stimulators of prostaglandin synthesis which demonstrates a close link between two major inflammatory systems (the complement and arachidonic acid cascades). In the field of cytokines, this group together with P. Krammer (Heidelberg) showed that gamma-interferon (IFN-γ) is not the only macrophage activating factor. It is apparent that cytokines and prostaglandins modulate each other's effect. This was recently demonstrated by studying synthesis of tumor necrosis factor α (TNF-α), which could be stimulated by increased PGE_2-dependent cyclic GMP, whereas cyclic AMP displayed suppressive effects[10]. Kaever and Resch (Hannover) are also involved in research on arachidonic acid metabolism. Their data suggest that protein kinase C is centrally involved in the regulation of macrophage eicosanoid synthesis via a phospholipase C-mediated signal transduction pathway. Thereby the intracellular free calcium level determines the metabolic route of liberated arachidonic acid[11].

Several groups are interested in the interaction of macrophages and lipopolysaccharides (LPS). Schade and Rietschel (Borstel) studied the endotoxin-induced formation of arachidonic acid metabolites and showed that macrophages release both cyclooxygenase and lipoxygenase products[12]. H-D. Flad (Borstel) and his group study the structure–function relationship between LPS and its fragments and their capacity to induce and modulate the production of interleukin 1 (IL-1) and TNF by human monocytes.

Freudenberg and Galanos (Freiburg) advanced knowledge of the interaction of LPS and macrophages by showing that macrophages responsive to LPS play a central role in the induction of LPS lethality, and also in tolerance to the lethal activity of LPS for galactosamine-sensitized mice[13].

Macrophage – lymphocyte interactions

At the Institute of Immunology (Mainz), A.B. Reske-Kunz, K. Reske, E. Rüde and their collaborators mainly study the role of macrophages and other cell types (IA^+ T-cell clones, IA-transfected L cells) in antigen presentation. Macrophages derived from bone-marrow cultures supplemented with granulocyte–macrophage colony-stimulating factor (GM-CSF) were induced by IFN-γ and GM-CSF to maximum antigen-presenting capacity[14]. Antigen processing is also studied using insulin and a number of its A-chain derivatives. All these antigens, including a 14 amino acid peptide, required processing for efficient presentation with no indication of an involvement of proteolysis. The processing of antigen but not the antigen-IA recognition was blocked by inhibition of *N*-glycosylation of proteins in antigen-presenting cells[15].

The work of J. Knop (Mainz) centers around the question of which cells participate in the induction of allergic contact dermatitis. This work also includes the role of Langerhans cells and other dendritic cells in the induction of cellular immune reactions. W. Klinkert (Würzburg) and his group have isolated rat dendritic cells from various lymphatic tissues[16]. These Fc receptor-negative dendritic cells act as accessory cells in mitogen-driven responses and present soluble antigen (acetylcholine receptor) to antigen-specific cell lines. These dendritic cells originate from radio-sensitive precursors in the bone marrow which can differentiate *in vitro* in the presence of a growth factor from supernatants of concanavalin A (ConA)-stimulated spleen cells.

H. Peters (Göttingen) has shown that human peripheral blood monocytes can be converted *in vitro* into highly potent accessory cells for T-cell activation. Concomitantly, they downregulate typical monocyte/macrophage markers, such as Fc receptors and nonspecific esterase, they express dendritic morphology and veils and may represent an ontogenetic link between monocytes and accessory cells in tissues.

Another approach to the study of macrophage–lymphocyte interaction

was taken by Hadding (Düsseldorf), who introduced the toxin of *Clostridium dificile* as a substance acting on macrophages to stimulate T-cell proliferation[17].

R. Andreesen and co-workers (Freiburg) study the functional and phenotypic changes that accompany the maturation of human macrophages from blood monocytes[18]. One of their aims is to generate autologous activated macrophages in culture for re-infusion into cancer patients.

Macrophages as effector cells

M.L. Lohmann-Matthes' group (Hannover) defined a new type of tumoricidal and microbicidal effector cell as an immature nonadherent precursor cell of the macrophage lineage[19]. This cell type has been isolated from bone marrow, spleen and liver of normal animals. It can be recruited in large numbers to these organs or can perform strong proliferation in an organ-associated way. IL-2 is a potent activator of this cell type and endows it with strong natural killer (NK)-like activities. Baccarini (Hannover) described a p75 IL-2 receptor on these cells as the only receptor responsible for the interaction with IL-2 (Ref. 20). Macrophage precursors are increased in number during infections or tumor diseases and represent an effector cell population which is easily manipulated.

Männel and co-workers (Heidelberg) were the first to describe TNF as a product of activated macrophages, thus representing a cytotoxic effector molecule of macrophages[21]. Recent evidence from this group shows that defined tumor cell membrane proteins can induce TNF production in human monocytes. T. Decker (Hannover) first reported the existence of membrane-associated TNF as a lytic mechanism of cell-mediated macrophage cytotoxicity[22]. Ziegler-Heitbrock (München) showed that human monocytes kill the TNF-sensitive target Wehi 164, both by means of secreted TNF and also apparently by TNF-dependent cell-mediated mechanisms[23]. The role of macrophages in the development of type 1 diabetes is studied by H. Kolb and co-workers (Düsseldorf). Macrophages precede lymphocytes in pancreatic inflammation areas and macrophage inhibition prevents islet inflammation and diabetes development. *In vitro*, macrophages of diabetic rats spontaneously lyse islet cells but not thyrocytes or hepatocytes. Particle phagocytosis by liver macrophages (Kupffer cells) is studied by V. Kolb-Bachofen's group (Düsseldorf), with particular emphasis on the role of carbohydrates in recognizing receptors and blood constituents as opsonizing agents[24].

Zawatzky and Kirchner (Lübeck) showed that resistance of inbred mouse strains to intraperitoneal infection with herpes simplex virus type 1 (HSV-1) is mediated by an early IFN response at the local infection site[25]. Resistance can be broken by neutralizing anti-IFN antibodies, injected simultaneously but not later than eight hours after infection. Using an *in-vitro* model, Domke-Opitz and Kirchner have shown that replication of HSV-1 is strongly inhibited in macrophage cultures from C57/B16 mice because of rapid release of IFN[26]. The data suggest that restriction of HSV-1 replication and spread *in vivo* is mediated by an early IFN response of macrophages.

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THEME TWO

The following three articles have separate authors but share a common theme and have been united under a general heading.

Autoimmunity

Mechanisms of autoantibody formation and chemically-induced autoimmunity

E. Gleichmann

Studies of murine graft-versus-host disease (GVHD) were started by E. and H. Gleichmann in R.S. Schwartz's laboratory at Tufts Medical School, Boston, and were continued in Hannover, Amsterdam and Düsseldorf. Using the parent into F_1 GVH model they were able to induce lymphomas, systemic lupus erythematosis (SLE) and other signs of collagen vascular diseases. In collaboration with A.G. Rolink, S. Pals, T. Radaszkiewcz, M. Kimura and others, they established that all these diseases are inducible by T cells and that autoreactive B cells capable of producing SLE-like autoantibodies are present in genetically normal animals. Autoantibody formation in the GVH model is not a random polyclonal process, but is biased towards production of IgG autoantibodies that are characteristic of SLE and other systemic autoimmune diseases. Furthermore, autoantibody formation in the GVH model of SLE is probably due to unlinked or bystander T-cell–B-cell cooperation. In this respect, autoantibody formation in systemic autoimmune diseases might fundamentally differ from that in organ-specific autoimmune diseases – such as myasthenia gravis – which appear to involve cognate T-cell–B-cell cooperation.

Evidence for such cognate T-cell–B-cell cooperation in organ-specific autoimmunity was obtained by R. Hohlfeld, K. Toyka and colleagues at the Department of Neurology, Heinrich-Heine-University of Düsseldorf. From a patient with myasthenia gravis they established a $CD4^+$ T-cell line specific for the acetylcholine receptor (AChR). When this line was cultured with the patient's monocytes and B cells in the presence of AChR, IgG autoantibodies to AChR were produced. However, there is also circumstantial evidence that is not readily consistent with the concept of bystander T-cell–B-cell cooperation as the mechanism of autoantibody formation in systemic autoimmunity, at least as far as small nuclear ribonucleoproteins (snRNP) are concerned. R. Lührmann and co-workers (Institute for Molecular Biology, University of Marburg) succeeded in producing IgG antibodies to the immunodominant domains of U1 snRNP and Sm following immunization of normal mice with native human U1–U6 snRNP and complete Freund's adjuvant; autoantibodies from patients with systemic autoimmune diseases react to closely related, if not the same, domains. Furthermore, using a very similar preparation of U1–U6 snRNP and peripheral lymphocytes of a healthy human donor, G. Wolff-Vorbeck and H-H. Peter (Department of Clinical Immunology, University of Freiburg) prepared a $CD4^+$ T-cell line that proliferates in response to U1–U6 snRNP, but not control antigens, and is restricted by the human leukocyte antigen (HLA) DR4.

The Gleichmanns and their colleagues proposed the hypothesis that GVH-like diseases might develop if T cells react to autologous major histocompatibility complex (MHC) structures that were rendered 'foreign' by viruses or drugs, such as phenytoin, D-penicillamine, and gold sodium thiomalate. In the mouse, experimental evidence consistent with this hypothesis was obtained by showing specific helper-T-cell reactions to these drugs. Because it is technically difficult to demonstrate T-cell reactions to small chemicals *in vitro*, the direct popliteal lymph node assay (PLNA) and the adoptive transfer PLNA in rodents were used. Together with C. Klinkhammer, M. Muranyi and H-W. Vohr (Düsseldorf) it was shown that the PLNA is a simple and reliable method for detecting T-cell sensitization to drugs and their metabolites during the early preclinical test phase.

In humans, T-cell sensitization to drugs has been shown by the groups of P.A. Berg (Department of Medicine, University of Tübingen) and H. Merk (Dermatological Clinic, University of Cologne), who both used the lymphocyte transformation test. Merk also succeeded in showing T-cell sensitization to drug metabolites. This was achieved by incubating the drug to be tested with liver microsomes as a source of cytochrome P450 – an enzyme system responsible for drug metabolism. Human antibodies specific for erythrocyte-bound drug metabolites were detected by C. Mueller-Eckhardt and co-workers (Institute for Blood Transfusion and Clinical Immunology, University of Giessen). They used '*ex-vivo* antigens' – i.e. plasma or urine of probands taken at appropriate times after drug administration.

At the University of Mainz, F. Oesch (Institute for Toxicology) and K-H. Meyer zum Bueschenfelde (Department of Internal Medicine) and their co-workers are investigating the pathogenesis of non-viral hepatitis. Studies pioneered by P. Beaune, Paris, established that patients suffering from drug-induced hepatitis produce autoantibodies against that particular isoform of hepatic cytochrome P450 that is responsible

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for metabolizing the inducing drug. Z. Amelizat *et al.* (Mainz) observed that autoantibodies against cytochrome P450 are a general phenomenon in patients suffering from idiopathic chronic hepatitis, suggesting that chemicals are involved in the etiopathogenesis of such cases.

Current research by E. Gleichmann's group (Medical Institute of Environmental Hygiene, Düsseldorf) focusses on the GVH-like autoimmune disease inducible by $HgCl_2$. Based on the work of P. Druet (Paris) and others, a mouse model has been established. Upon treatment with $HgCl_2$, susceptible strains develop high serum IgG and IgE levels and extremely high titers of antinucleolar autoantibodies (ANolA). In association with R. Lührmann's group (Marburg) fibrillarin, a component of U3 snRNP, and an snRNP of more than 90 kDa were identified as the major antigens detected by these AnolA. Very similar, if not identical, autoantibodies occur in idiopathic scleroderma and GVH mice. Injection of anti-interleukin 4 (IL-4) into $HgCl_2$-treated mice abrogated the enhanced IgE formation, indicating that $HgCl_2$ activates the helper T cell subpopulation, T_H2 (M. Ochel and H-W. Vohr). Susceptible mouse strains treated with $HgCl_2$ contain T cells primed to $HgCl_2$ as the nominal antigen. This finding is consistent with a GVH-like pathogenesis of $HgCl_2$-induced autoimmunity. The MHC plays a major role in determining susceptibility to the $HgCl_2$-induced autoimmune disease. While susceptibility to ANolA formation is determined by $I\text{-}A^s$, concomitant expression of I-E significantly suppresses it. Thus, this response to self-antigen follows the rules established for responses to nonself-antigens, as established by C. Baxevanis, Zoltan Nagy and Jan Klein (Max-Planck-Institut for Biology, Tübingen) and evident in a variety of murine parasite infections (Chella David, Rochester), including *Plasmodium chabaudi* (F. Wunderlich, Division of Parasitology, Heinrich-Heine-University of Düsseldorf).

Clinical autoimmunity

J.R. Kalden

Owing to new combined efforts made by the Deutsche Forschungsgemeinschaft, the Max-Planck-Gesellschaft and the Ministry for Research and Technology, including new programs to improve clinical science in the FRG, research activities in clinical autoimmunity have improved considerably. In the field of neuroimmunology, the role of the nicotinic acetylcholine receptor and distinct antigenic epitopes in the pathogenesis of myasthenia gravis have been studied by K. Toyka and his group[1,2]. While studying the involvement of the thymus clone in the pathogenesis of myasthenia gravis, Müller-Hermelink's group showed acetylcholine-receptor-related antigenic determinants in tumor-free thymuses and thymic epithelial tumors[3,4], supporting the notion that the thymus might be involved in pathogenic events leading to myasthenia gravis. Worldwide studies of the pathogenesis of myasthenia gravis, including its immunogenetics[5], have now been started, to develop immunological therapeutic strategies to improve the present therapeutic regimes for this disease.

Immunohematology research here has concentrated on the identification of platelet reactive antibodies[6] as well as on the definition of alloantigen systems on platelets and their clinical relevance for idiopathic thrombocytopenia[7,8]. The involvement of cold agglutinins in the pathogenesis of autoimmune hemolytic anemias has been investigated by D. Roelcke *et al.*[9].

In the interesting field of the interaction between the endocrine and the immune system, studies are in progress on the immunopathogenesis of type I diabetes and its possible immunotherapy[10], its serological markers[11], the description of proinsulin-specific autoantibodies[12], and finally data on the immunogenetics of type I diabetes[13]. In other areas of endocrine disorders recent interesting experiments performed by Scriba *et al.*[14] provide evidence that plasmid encoded proteins from enteropathogenic *Yersinia* might play a role in the onset of Graves' hypothyroidism.

Immunologically-mediated liver disease research has concentrated on the involvement of immunological regulating mechanisms with special emphasis on disease entities such as acute and chronic hepatitis B and primary biliary cirrhosis (PBC). Immunohistological analyses of mononuclear cells infiltrating the liver as well as T-cell clones obtained from liver tissue and from peripheral blood showed that in areas of tissue destruction in chronic hepatitis B, $CD8^+$ T cells were found, while (natural killer) NK or K cells were not. Similarly, in PBC, $CD8^+$ T cells were found surrounding the bile ducts. Established T-cell clones from liver tissue expressed CD8 markers in hepatitis B, in contrast to PBC where cytotoxic T cells expressed both the CD8 and the CD4 molecules. In further studies, data were obtained indicating that the epithelial cells of bile ducts are the target of autoimmune reactions[15,16].

Meyer zum Büschenfelde's group in Mainz, working in the same area, has made significant contributions with regard to target antigens of autoimmune attack on hepatocytes. They identified the major target of liver and kidney microsomal autoantibodies in idiopathic autoimmune hepatitis as cytochrome P450 db1 (Refs 17, 18). They are now evaluating the effect of alpha-interferon treatment of hepatitis B (Ref. 20).

Immunologically-mediated kidney diseases are studied in different centers in the FRG. Remarkable data have been collected by Weber *et al.* (formerly in Mainz and now in Erlangen) with regard to the identification of the target antigens of autoantibodies in Goodpasture's syndrome[21,22]. Similar research activities are being carried out by

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Rauterberg's group in Heidelberg[23,24]. Studies are now in progress to find out why collagen type IV, which is a common constituent of the extracellular matrix, acts only in the Goodpasture syndrome as a target for an organ (kidney and lung)-specific autoimmune reaction. Further research activities are concentrating on the role of complement in immunologically-mediated kidney diseases[25].

Owing to a special program of the Ministry for Research and Technology, research on the pathogenesis of rheumatic disease entities has made considerable progress. Four centers are mainly involved: the Medical School in Hannover, the University of Heidelberg, the University of Freiburg and the University of Erlangen-Nürnberg. Hannover is the focus of a multicenter study aimed at establishing the involvement of immunogenetic factors, for example the association with major histocompatibility complex (MHC) class I, class II and class III antigens, and haplotypes on a large number of systemic lupus erythematosis (SLE) patients. In addition, a Sonderforschungsbereich of the Deutsche Forschungsgemeinschaft has been established in Hannover, dedicated to clinical and molecular biological investigation of chronic inflammatory mechanisms leading to destruction of joints. With regard to immunopathogenic mechanisms involved in chronic inflammatory joint diseases, research activities in Heidelberg are concentrating on molecular mechanisms of the genetic predisposition in rheumatoid arthritis (RA) patients, including research on arthritis antigen-specific stimulation of T cells and T-cell clones against disease-associated MHC-determinants[26,27]. In cooperation with the Max-Planck-Institut für Immunologie, the Department for Rheumatology and Clinical Immunology in Freiburg is working on, among other topics, autoreactive T cells in rheumatic diseases. Special emphasis has been focused on Lyme arthritis[28,29].

Research programs in Erlangen, at the Max-Planck Clinical Research Group, associated to the Institute for Clinical Immunology and Rheumatology, study the phenotypes of T cells and macrophages in the inflammatory synovium of patients with RA, the search for clonally expanded T cells in RA, and investigate the immune response to cartilage antigens and to related microbial antigens[30,31]. In the same center, studies are underway to look for the interaction of cells of the immune system with chondrocytes – characterizing antigenic determinants on chrondrocytes including the establishment of T-cell clones specific for chrondrocyte membrane antigens. These studies also include the evaluation of cytokines in activating mechanisms of mesenchymal cells of the inflammatory altered synovial membrane. Experiments to modulate cell surface antigens of the mononuclear phagocytic system by different cytokines are in progress. Finally, T-cell clones obtained from the inflamed synovial membrane of RA patients will be analyzed with regard to their possible antigen specificity using a 150 kDa protein obtained from the synovial fluid of RA patients[32–34].

Studies on the pathogenesis of reactive arthritides are being conducted by Bitter-Suermann in Hannover, Heesemann in Hamburg, as well as in Erlangen and Munich[35], with a view to gaining insight into the pathogenic mechanisms leading to RA. Ongoing research activities with regard to clinical autoimmunity in rheumatic diseases also include new approaches to therapy. Thus, in various centers, for example Heidelberg, Erlangen and Munich, in cooperation with Basle, a treatment schedule has been started using anti-CD4 antibodies in the treatment of patients with RA refractory to the traditional therapeutic regimes. Also in RA, as in other chronic connective tissue diseases, treatment attempts have been started by using different cytokines such as gamma-interferon, for RA as well as for patients suffering from systemic progressive sclerosis.

Coming back to research on SLE, two lines of activities are obvious: first, experiments with regard to the etiopathogenesis of SLE and, second, research on the molecular biology and the antigen cloning of nuclear and cytoplasmic antigens serving as target antigens in different connective tissue diseases, besides SLE.

Addressing the problem of the oligo- or monoclonal origin of anti-dsDNA antibodies, research in Erlangen showed that antibodies against dsDNA in SLE patients are of oligoclonal origin[37] and, furthermore, that a patient-specific antinuclear antibody pattern is present[38]. New data also suggest that retroviral structures might possibly be involved in the pathogenesis of SLE. Studies conducted by Krapf *et al.* support the idea of retroviral involvement in the triggering events leading to SLE[39,40].

Further research activities, for example by Luermann, concentrate on the cloning and sequencing of U1 RNPs; this antigen is also considered important as a target antigen in SLE. Experiments on antigen cloning in other chronic rheumatic diseases such as Wegener's granulomatosis, juvenile RA, polymyositis, and progressive scleroderma, as well as overlap syndromes[41–45] are also in progress.

It should also be stressed that several institutions in the Federal Republic are involved in the international Standardization Efforts for Reference Material for the Demonstration of Autoantibodies. Thus, reference material was obtained for different autoantibodies against nuclear antigens[46], islet cell[47] and acetyl receptor antibodies[48].

Finally, data were obtained recently regarding dilated cardiomyopathy suggesting that at least part of this disease is due to an autoimmune reaction[49].

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Immunology and diabetes – on the search for triggers and effectors

Helga Gleichmann

It is widely believed that insulin-dependent diabetes mellitus (IDDM) in humans is a sequela of chronic pernicious autoimmune reactions resulting in progressive destruction of β cells of the pancreatic Langerhans islets. Hypothetically, in genetically susceptible individuals as yet undefined factors could initiate immunological reactions antedating loss of β cells. Aberrant expression of major histocompatibility complex (MHC) class II molecules on β cells has been proposed by Gian Franco Bottazzo and his colleagues (London) to permit presentation of β-cell-specific antigens for autoimmune recognition as the initiating event. However, a direct autoimmune attack on β cells has still to be unequivocally proven.

There are three animal models that can be used to elucidate the effector mechanisms of gradual β cell destruction associated with metabolic and immunological characteristics corresponding to those of IDDM in humans. These are first, the spontaneously developing diabetic syndromes of the BB (Bio-Breeding) rat; second, the NOD (Non Obese Diabetic) mouse and third, the form chemically-induced by multiple injections of streptozotocin (STZ) – an *N*-nitroso derivative of D-glucosamine, in susceptible mouse strains. There is general worldwide agreement that T cells are involved in the pathogenesis of diabetes in all three models and that histologically there is insulitis, i.e. infiltrates of mononuclear cells at peri-islet and intra-islet sites. Again, evidence for a direct autoimmune attack of unaltered rodent β cells still has to be shown. At the Diabetes Research Institute at the Heinrich-Heine-University of Düsseldorf, investigators are exploring the mechanism(s) underlying the immune reactions involved in diabetes development in animal models.

Helga Gleichmann and her associates analyse the specificity and significance of immune reactions involved in β-cell-destructive processes and the initial site of action of STZ. They showed that T cells can be specifically stimulated by STZ. This was shown by means of the popliteal lymph node (PLN) assay. A T-cell-dependent primary immune response towards STZ could be induced and specific secondary PLN responses developed in STZ-primed recipients. In adoptive transfer experiments, STZ-sensitized splenic T cells were detected. More recently, Christiane Klinkhammer found that STZ can readily induce transient T-cell-dependent MHC class II antigen expression in cells of multiple organs including the pancreas. Pancreatic

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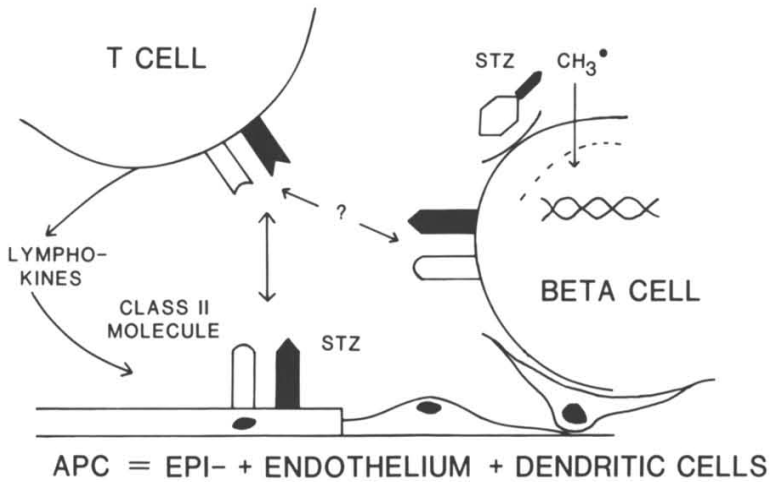

Fig. 1. *Postulate of STZ recognition by T cells in the pancreas. T-cell reactions are induced by STZ attachment to MHC class II molecules of antigen-presenting cells (APC) of epithelial (EPI-), endothelial and dendritic cells present at the islet pole and at intra-islet sites. I-A positivity might be upregulated upon local T-cell-dependent lymphokine release resulting in increased lymphocyte reactivity.*

endocrine cells, however, remained devoid of class II antigens. Figure 1 illustrates a presumed sequence of STZ-induced reactions. T_H cells can be specifically stimulated by STZ attached to class II molecules on antigen-presenting cells (APCs) of ductular epithelium and vascular endothelium of the pancreatic islet pole as well as dendritic cells adjacent to intra-islet capillary walls. Upon local release of T-cell-dependent lymphokines, upregulation of cellular class II antigen expression occurs, increasing T-cell reactivity and recruitment of inflammatory cells. These reactions might enhance subtoxic effects on β cells exerted by the alkylating activity of the methylnitrosourea moiety of STZ. Stimulation of T-cell responses by modified β cells, however, remains questionable in this animal model of diabetes.

In cooperation with Bernard Green at the Hebrew University in Jerusalem, we are comparing a number of newly synthesized STZ analogues with respect to their diabetogenicity and immunogenicity.

Hubert Kolb and his group are studying the role of macrophages in the disease process of BB rats and STZ-induced hyperglycemia in mice. In both models, ultrastructural studies demonstrated discrete intra-islet infiltration by macrophages preceding diabetes manifestation (Victoria Kolb-Bachofen). Administration of silica, which can inhibit macrophage activity almost completely prevented disease development in these animal models. Accordingly, macrophages were assigned an essential role in the early phase of the disease process. They suggested that prevalence of macrophages able to engulf islet cells in the pre-diabetic phase indicates their cytotoxic activity towards β cells. Currently, they are analysing mechanism(s) of macrophage-mediated islet cell lysis *in vitro*. Their data on the preventive effect of T-cell depletion and/or inactivation on diabetes development in IDDM animal models are in agreement with those reported by other laboratories. In addition, the effect of various immunomodulating compounds on the disease process is under investigation, with a view to designing a therapeutic regimen applicable in clinical trials.

Thomas Linn and Konrad Federlin at the Clinical Department of the Justus Liebig University of Gießen are also engaged in defining the sequence of events leading to β-cell destruction in the STZ model. They found an STZ-induced prevalence of intra-islet macrophages at early disease stages, and increased MHC class II antigen expression on infiltrating mononuclear cells, but not on β cells. Another aspect of their research is to abrogate the β-cell-destructive process by dietary compounds such as ω-3 polyunsaturated fatty acids.

George Köhler and his colleagues at the Max-Planck-Institute for Immunology in Freiburg are studying the mode of tolerance versus autoaggression towards β cells by using transgenic mice. Their first data indicate that MHC class II antigen expression on β cells results in loss of insulin secretion. Signs of lymphocyte activation, however, were not obtained. These results are in agreement with those of Sarvetnick *et al.* (San Francisco) and Lo *et al.* (Philadelphia). Expression of MHC class II antigens was directed by linking the respective DNA sequences to the rat insulin promoter. Presumably, the pathogenesis of diabetes in these transgenic mice is not immunological in nature.

T. Diamantstein and his coworkers at the Immunology Research Unit of the Free University of Berlin with Hans Jürgen Hahn from Karlsburg, GDR, are focussing on abrogation of β-cell destruction in BB rats by transient treatment with immunosuppressive agents. This goal has been achieved with combined therapy of the anti-IL-2 receptor mAb, ART 18 and a subtherapeutic dose of cyclosporin A. This regimen resulted in prolonged euglycemia in BB rats when given immediately after diabetes manifestation and in survival of allogenic β-cell grafts in LEW.1W rats rendered diabetic by streptozotocin. Currently, they are analysing whether this effect is due to elimination of reactive T lymphocytes only or if a state of tolerance has been induced as well. Their studies could lead to development of intervention and/or substitution therapy for patients with diabetes.

Tumor immunology: Paul Ehrlich's heritage

Judith P. Johnson[1], Gert Riethmüller[1] and Volker Schirrmacher[2]

The foundations of tumor immunology can be traced back to the work of Paul Ehrlich who, at the turn of the century, proposed that tumors express structures that can be recognized as foreign by the host, and that this immune surveillance is responsible for the relatively low frequency of tumors. "Sonst müßte das Karzinom in geradezu ungeheuerlicher Frequenz auftreten"[1]. Ehrlich established tumors which could be maintained by transplantation in animals and attempted to show tumor immunity. However, in the absence of knowledge of transplantation antigens, results were irreproducible and Ehrlich left this work in the hands of his assistant Hugo Apolant. G. Schöne – who with Ehrlich immunized rabbits with embryonic tissue prior to transplanting sarcomas – quotes Paul Ehrlich as having stated that the problem of cancer is too difficult. "Krebs ist zu schwer"[2]. With the death of Apolant in the first year of World War I, work on tumor immunology in Ehrlich's institute stopped. Today, nearly a century after his proposal, both tenets of tumor immunology, that is that spontaneous tumors express unique or tumor-specific antigens and that they are effectively recognized by the host, remain largely unproven. Nevertheless Paul Ehrlich's heritage can be found in an active contribution by German immunology to the study of these questions and to the application of the immune system in tumor therapy.

The identification of tumor-associated antigens

The immune system is a sensitive discriminator of structural variation and has been extensively used to search for differences between tumors and normal cells. In the 1960s this approach led to the identification of a number of oncofetal antigens that appeared to be expressed selectively by human tumors. Among these was the carcinoembryonic antigen (CEA), which continues to be widely used to monitor the course of disease in tumor patients and is now known to encompass a family of related molecules, several of which have been cloned (S. von Kleist, P. Zimmermann, J. Thompson, F. Grunet, Freiburg)[3].

The development of monoclonal antibody (mAb) technology infused new enthusiasm into the search for tumor antigens and in recent years many new tumor-associated molecules have been identified on human tumors (gastrointestinal tumors: W. Dippold, K-H. Meyer zum Büschenfelde, Mainz; H.P. Vollmers, H.K. Müller-Hermelink, Würzburg; K. Bosslet, H.H. Sedlacek, Marburg; J. Johnson, G. Riethmüller, Munich; melanoma: E. Bröcker, E. Macher, C. Sorg, Münster; J. Johnson, G. Riethmüller, Munich; bladder carcinoma: F. Falkenberg, Bochum; renal cell carcinoma: J. Scherberich, Frankfurt; F. Oesch, Mainz; bronchial carcinoma: C. Gropp, K. Havemann, Marburg; lymphoma: H. Stein, J. Gerdes, Berlin; V. Diehl, M. Pfreundschuh, Cologne). In addition to providing information about biochemical changes occurring during malignant transformation, these antibodies can be used in tumor diagnosis, in monitoring disease course and in therapeutic trials. MAbs directed to leukocyte differentiation antigens (B. Dörken, G. Moldenhauer, R. Schwartz-Albiez, Heidelberg; E. Thiel, Berlin; M. Gramatzki, Erlangen; P. Rieber, Munich; A. Feller, H. Radzun, Kiel) have also been useful in the diagnosis and characterization of leukemias and lymphomas. One of the approaches that is widely used to determine the tissue origin of carcinomas and their metastases is the definition of their reactivity with anti-cytokeratin antibodies[4]. The development of this approach resulted from intensive collaboration between the groups of W.W. Franke (Heidelberg) and K. Weber and M. Osborn (Göttingen), who identified and characterized the cytokeratins expressed by various tissues. Recently, it has been possible to detect bone-marrow micrometastases using anti-cytokeratin antibodies that can distinguish the tumor cells (which are of epithelial origin) from the bone-marrow cells and stroma. The presence of such micrometastases in patients with no other sign of metastatic disease is predictive of the development of frank metastatic disease[5].

The role of cell adhesion molecules in tumor growth, invasion and metastasis

The development of a fully metastatic tumor is a complex, extended process proceeding through different distinguishable stages and involving activation or inactivation of a number of different genes. MAbs produced against tumor antigens are useful in the dissection of these various stages and in the identification of the molecular changes that characterize them. In experimental models, mAbs have been isolated that inhibit the growth of tumor cells both *in vivo* and *in vitro* and modify their invasive behavior (W. Birchmeier, Essen)[6]. The antigens detected by such antibodies have frequently been found to be directed against molecules which play a role in intercellular adhesion, for example L-CAM/uvomorulin.

The analysis of related tumor cell lines differing in their *in-vivo* metastatic capacity is one approach that is used to identify the molecular changes that are associated with the development of metastatic capacity[7]. The production of mAbs distinguishing between high and low metastatic variants provides tools with which to identify biochemical differences and to isolate metastasis-associated genes from expression libraries (U. Günthert, Karlsruhe). A low metastatic lymphoma variant which showed increased *in-vitro* adhesion differs from its highly metastatic counterpart in a number of characteristics including the aberrant expression of the neural adhesion molecule L1 (P. Altevogt, Heidelberg). Lectins have also been identified on tumor cells and their interactions with carbohydrate determinants on other cells may also contribute to the adhesive properties which are critical to tumor–host in-

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teractions (H.J. Gabius, Göttingen; G. Uhlenbruck, Cologne; V. Schirrmacher, Heidelberg)[8].

The identification of molecules associated with tumor progression in human tumors has been approached by looking for mAbs that show reactivity patterns on frozen tissue sections that correlate with clinical parameters predictive of the development of metastasis. This approach has led to the identification of several molecules potentially associated with malignant transformation and metastasis formation in human cutaneous melanoma (E. Bröcker, C. Sorg, E. Macher, Münster; J. Johnson, G. Riethmüller, Munich). Two molecules expressed by melanomas with a high risk of metastasis have been cloned; one (MUC18) is a cell surface molecule belonging to the immunoglobulin superfamily and showing sequence similarity to a group of neural cell adhesion molecules and to CEA. The second molecule is the intercellular adhesion molecule ICAM-1 (Ref. 9). A molecule whose expression is associated with malignant transformation in a variety of human tumors has also recently been shown to be a cell adhesion molecule (E. Klein, Ulm). Thus a variety of different adhesion molecules may play roles in the development and progression of malignancies. The lymphocyte homing receptors with differing organ specificities may also be involved and recent studies show that they can be selectively regulated upon cell activation (A. Hamann, Hamburg).

Tumor immunity and escape mechanisms

The presence of specific tumor-reactive lymphocytes in tumor patients can be shown by cloning cells isolated either from the tumor itself or from the peripheral blood (T. Wölfel, A. Knuth, Mainz; E. Schneidar, P. Wernet, Tübingen). The analysis of such autologous cytotoxic T lymphocytes (CTL) clones from melanoma patients suggests that a variety of distinct antigens are recognized by T lymphocytes[10] and in one case the human leukocyte antigen, HLA-A2, has been identified as the restriction element (T. Wölfel, A. Knuth, Mainz).

Tumor-specific CTL or their precursors can be detected concurrently with a progressively growing tumor suggesting that the tumor is able to escape from effective control by the immune system. The loss of major histocompatibility complex (MHC) restriction elements may be one way in which tumors can escape immune destruction. In animal models, the loss of particular MHC allele expression is associated with progressive tumor growth and metastasis development and this can be reversed by transfection of the missing gene (G. Hämmerling, Heidelberg)[11]. In another animal tumor model, a tumor-antigen-negative, CTL-resistant, immune escape variant could be shifted to a tumor-antigen-positive, CTL-sensitive line by treatment with the DNA-demethylating drug 5-azacytidine[12].

Variation in the expression of MHC antigens has also been observed on human tumors. HLA class I antigens are frequently lost, as for example in colorectal carcinomas (F. Momburg, Heidelberg) where this is due to the lack of β_2-microglobulin mRNA expression[13], while HLA class II molecules are newly expressed. In a prospective study of malignant melanomas, the expression of HLA class II antigens was a marker for poor prognosis and the early development of metastases (E. Bröcker, E. Macher, C. Sorg, Münster)[14]. The observation that variations in the expression of HLA antigens have prognostic value may indicate that the immune system does indeed play a role in the growth and development of spontaneous human tumors. Various groups are studying the mechanisms of activation of natural killer (NK) cells (for example, R. Schmidt, Hannover), macrophages (for example, M.L. Lohmann-Matthes, Hannover; D. Gemsa, Marburg; H. Ziegler-Heitbrock, Munich), and T cells (S. Meuer, Heidelberg; T. Diamantstein, Berlin) and the role of lymphokines in these processes (H. Wagner, Ulm; H. Kirchner, Lübeck; P.H. Krammer and D. Männel, Heidelberg; K. Pfitzenmaier, M. Krönke, Göttingen).

Another approach to the role of the immune system in tumors is to identify functionally distinct populations of mononuclear cells comprising the tumor infiltrate and to examine changes in this population that accompany tumor progression (E. Bröcker, C. Sorg, Münster; J. Kalden, Erlangen). In animal model systems, it appears that T-cell mediated responses have a higher anti-tumor protective capacity than NK mediated responses (M. Zöller, Heidelberg)[15] and suppressor T cells play a role in at least some cases. In a murine plasmacytoma model, one of the earliest events to be detected is the activation of tumor-specific suppressor cells which prevent the induction of specific CTL against this immunogenic tumor (J. Heuer, E. Kölsch, Münster)[16].

Immunological approaches to tumor therapy

Paul Ehrlich realized the uniqueness of immunological reagents for therapy when he dubbed them 'magic bullets', noting that they seek out and destroy their targets without damaging the organism[2]. The recent advances in cellular immunology together with molecular cloning of many of the lymphokines have significantly improved the prospects of establishing an effective immunological approach to tumor therapy. A number of approaches to immunotherapy of tumors are presently being evaluated in experimental systems, as well as in clinical trials. Treatment with biological response modifiers such as tumor necrosis factor α (TNF-α) (M. Pfreundschuh, V. Diehl, Cologne; G. Nagel, M. Krönke, K. Pfitzenmaier, Göttingen), and interleukin 2 (IL-2) (R. Mertelsmann, Mainz) is being used in a number of different tumors. In several animal models, immunization with autologous tumor cells which have been modified by mutagenesis or viral infection results in an anti-tumor immune response that is also effective against metastasis of the unmodified tumor cells[17]. This active specific immunotherapy is currently being used in clinical trials with autologous tumor cells modified by infection with Newcastle Disease virus (V. Schirrmacher, Heidelberg) or by mutagenesis (A. Knuth, Mainz). MAbs allow passive targeting of tumor cells and are being used in clinical trials against colorectal carcinoma (mAb 17-1A, I. Funke, G. Riethmüller, Munich) and malignant melanoma (R24, W. Dippold, K-H. Meyer zum Büschenfelde, Mainz)[18]. Individually prepared anti-idiotypic antibodies are being used to treat myeloma patients (G. Moldenhauer, Heidelberg). A refinement is to use mAbs to target cytotoxic reagents, such as radionuclides, toxins, drugs or even cytotoxic cells to the tumor (S. Matzku, Heidelberg: K. Bosslet,

H. Sedlacek, Marburg; G. Jung, Munich)[19,20]. The potential of these conjugates in immunotherapy is an active area of current research and the influence of Paul Ehrlich appears again since he discovered the lectins abrin and ricin, two of the plant toxins frequently used to construct them.

"Krebs ist zu schwer." The problem of cancer appears as difficult today as it did to Paul Ehrlich. Nevertheless, technological advances in cloning and maintaining in culture both antibody-producing cells and antigen-specific T lymphocytes have made it possible not only to study tumors and the host's immune response to them, but also to develop immunological approaches to the treatment of malignant tumors. The harnessing of the immune system for the destruction of tumor cells remains the heritage of Paul Ehrlich to all tumor immunologists.

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Immunity to infection

Stefan H.E. Kaufmann[1] and Martin Röllinghoff[2]

Parasites

Over the past ten years immunoparasitology has changed from a low technology endeavour to one at the forefront of the biotechnology revolution. Until then protozoan and helminth parasites – with their great impact on humans and some domestic animals – had largely resisted measures of control. When it became clear that the tools and concepts of cellular immunology, immunoregulation and molecular biology could be used in the analysis of complex host–parasite interactions, several groups in the Federal Republic of Germany joined the global research efforts to gain deeper insights into this field. This research is dominated by attempts to identify and analyse immune responses that are host-protective or parasite-inhibitory and to isolate and produce target antigens of such responses.

J. Langhorne and co-workers (Freiburg) are studying the immune response to *Plasmodium chabaudii* in mice. They have shown that CD4+ T cells are essential for successful parasite clearance *in vivo*[1] and have found have an inverse relationship between the frequencies of gamma-interferon (IFN-γ), interleukin 2 (IL-2)-producing T_H1 helper cells and T_H2 cells promoting antibody production – with a predominance of T_H1 cells early in the infection.

A. Crisanti, F. Sinigaglia and H. Bujard (Heidelberg) are developing a vaccine against the asexual blood stages of *Plasmodium falciparum*. Recently, they identified two invariant T-cell epitopes within the conserved part of the 190 kDa glycoprotein of *P. falciparum* merozoites that are recognized by both human T-cell clones and peripheral blood mononuclear cells from individuals infected with malaria[2].

Immunological and molecular aspects of the host–parasite relationship in murine cutaneous leishmaniasis are being studied by C. Bogdan, M. Lohoff, W. Solbach and M. Röllinghoff (Erlangen). They have shown that cyclosporin A has a suppressive effect on infection with *Leishmania major* by inhibiting the expansion of CD4+ T cells, thereby reducing the production of lymphokines such as granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-3, which are involved in the expansion of monocytes and macrophages that form the cellular basis of cutaneous lesions induced by *L. major*[3]. In addition, this drug enhances the elimination of the intracellular parasites by macrophages[4]. These observations are supported by the demonstration that recombinant GM-CSF has a detrimental effect on the course of the infection in BALB/c mice[5]. A second area of interest of this group is the functional analysis of the CD4+ T-cell subtypes that are instrumental in the defense action of the host. T cells with characteristics not only of T_H1 and T_H2 subtypes, but also of an intermediate type, have been cloned from BALB/c mice infected with *L. major*; this suggests

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that a critical balance of these functionally different T cells is required to overcome the infection. Furthermore, T_H2 cells specific for *L. major* have been shown to be responsible for the polyclonal B-cell stimulation and hypergammaglobulinemia that is typically found in chronic infections with *L. major*[6]. Recently H. Moll joined this group and is studying the T-cell immune response to the purified lipophosphoglycan of *L. major*.

D. Russell (Tübingen) also works with *Leishmania*. He has purified the abundant promastigote surface antigen gp63 and the lipophosphoglycan from *L. mexicana* and reconstituted them into liposomes. These liposomes act as a potent vaccine: in CBA and BALB/c mice they induce appreciable levels of protection[7].

M.L. Lohmann-Matthes and colleagues (Hannover), who study macrophages and their effects, have recently shown that macrophages and their non-phagocytic precursors can lyse *L. donovani* parasites extracellularly. She proposes that this spontaneous leishmanicidal activity may be of particular importance in the early phase of infection with *Leishmania*[8].

R. Williams and D. Dobbeleare (Karlsruhe) study lymphocytes transformed by *Theileria parva*. They have analysed the control of expression of genes related to T-cell proliferation, which are expressed while the cell is infected with the parasite, and have provided evidence that IL-2 and its receptor are constitutively expressed in cells infected by *Theileria*[9].

The immunology of schistosomiasis is studied by A. Ruppel, E. Beck and colleagues (Heidelberg) in three schistosome species, and their studies include experimental infections and work with schistosomiasis patients. One of their main aims is to develop a serological test on the basis of recently cloned antigens[10]. Research into a vaccine against *Schistosoma japonicum* is also being pursued, particularly in domestic animals.

R. Lucius (Heidelberg) is interested in the immunobiology of filarial infections in a rodent model and in human onchocerciasis – in particular, mechanisms regulating the population density of the parasites in the host. Surface antigens of infective stages and immunodominant secretory antigens of the parasites have been characterized by monoclonal antibodies (mAbs)[11]. An immunodominant antigen of *Onchocerca volvulus* was produced by molecular cloning and is currently being tested for its immunodiagnostic potential[12].

Bacteria

Interest in immunity to bacterial infections has a long history in Germany, and indeed many of the founders of this discipline were German. Nowadays several bacterial infections are well controlled – some of them as a result of immunological studies. Interest in antibacterial immunity remains high, especially as several infections still cause major health problems because of their intimate relationship with the immune system.

Several groups are now trying to define the relevant epitopes of bacterial components using synthetic peptides and/or mAbs in an attempt to develop diagnostic and therapeutic reagents. D. Bitter-Suermann, M. Frosch and co-workers (Hannover) succeeded in raising mAbs against the weak polysaccharide capsule antigen of *Escherichia coli* K1 and *Neisseria meningitidis* group B by using the autoimmune NZB mouse strain[13]. Although these mAbs are valuable diagnostic and therapeutic agents, they also react with the embryonic form of NCAM, an important cell adhesion molecule in the host[14]. The crossreactivity between bacterial and host-derived molecules could account for the poor immunogenicity of the capsule antigen. M.A. Schmidt (Heidelberg) is characterizing the binding of *E. coli* adherence factors. Immunization with synthetic peptides corresponding to certain epitopes of p-specific pili induces protection against infection with uropathogenic *E. coli* in a murine pyelonephritis model[15]. E. Jacobs, W. Bredt and co-workers (Freiburg) are analysing the binding sites of the *Mycoplasma pneumoniae* adhesion molecules[16].

Several investigators including V. Brade (Erlangen), A. Vogt (Freiburg), M.M. Simon (Freiburg), M.D. Kramer (Heidelberg) and B. Wilske (Munich) are interested in the newly emerging pathogen, *Borrelia burgdorferi*. When several *B. burgdorferi* strains were analysed by physicochemical and immunological methods, antigenic differences were seen between European and North American strains, which may be related to differences in the clinical pictures of Lyme disease[17].

S. Bhakdi (Giessen) has a long-standing interest in cytolytic effector molecules, immunity and infection – including not only the mechanisms by which the terminal complement components lyse target cells (including bacteria), but also the interaction of bacterial cytolysins with host cells. This group identified the *Escherichia coli* hemolysin as the most potent leukocidin known[18]. In addition, they characterized several ways in which *Staphylococcus aureus* α-toxin could induce pathological cell reactions[19].

Experimental *Listeria monocytogenes* infection in mice is being used by many groups for analysing intracellular bacterial infections in immunity. W. Goebel and his collaborators study virulence factors of this organism and have recently shown the importance of listeriolysin for intracellular survival[20]. H. Hahn and co-workers (Berlin) study the *in-vivo* role of CD4 and CD8 T lymphocytes in experimental listeriosis of mice. Their experiments indicate that in this model, $CD8^+$ T cells are primarily responsible for acquired resistance and granuloma formation, while $CD4^+$ T cells seem to be of major importance for delayed type hypersensitivity[21].

Various facets of cell-mediated immunity to bacterial and fungal infections are the major focus of S.H.E. Kaufmann's group in Ulm. The activation of antibacterial and antifungal macrophage effector mechanisms by interleukins is being studied using murine bone-marrow-derived macrophages as a defined cell population (summarized in Ref. 22). They have shown that in addition to interleukin-producing $CD4^+$ T cells, $CD8^+$ T cells with cytolytic activity are also involved in protection against intracellular bacteria (reviewed in Ref. 23). These findings led to the concept that cytolytic $CD8^+$ T cells may contribute to host resistance by releasing bacteria from their niches within host cells that are unable to destroy their intracellular parasites. In this way, more potent phagocytes could eliminate the pathogens. At the same time, lysis of host cells by $CD8^+$ T cells could be detrimental to the host since it allows microbial dissemination and leads to tissue destruction. Another

project of this group is the characterization of mycobacterial T-cell antigens of potential value for vaccine design against leprosy and tuberculosis. One of the major T-cell antigens, the 65 kDa protein, is a heat shock protein which is highly conserved (for summary see Ref. 24). T cells specific for this molecule recognize epitopes shared by various microbes, and could therefore participate in cross-immunity to different pathogens[25]. On the other hand, some T cells are directed to epitopes shared by the bacterial and human 65 kDa protein, and could be responsible for certain autoimmune sequelae of bacterial infections (reviewed in Ref. 25).

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Immunity to viruses

Matthias J. Reddehase

An understanding of how viruses and their hosts interact can be reached only by an interdisciplinary approach. Without considering the immune response of the host, the virologist cannot explain viral pathogenesis, and by viewing viruses as passive protein antigens, the immunologist cannot explain the immune response to infection. This brief overview on viral immunology in the Federal Republic of Germany cannot do justice to all who work in this field and – rather than reviewing merits of the past – it will focus on current basic research that is aimed at answering open questions about the mechanisms of protective immunity and immunopathology in viral infections.

LCM virus – questioning the antiviral role of cytolytic activity

F. Lehmann-Grube (Hamburg) is undoubtedly an important figure in the field of viral immunology in Germany. The main issue he and his collaborators D. Moskophidis, J. Löhler *et al.* address are mechanisms of recovery from acute lymphocytic choriomeningitis (LCM) virus infection. Lehmann-Grube made the surprising finding that few LCM virus-specific T lymphocytes of the CD8 subset showed a marked reduction of virus load in the spleen of infected mice within hours, without histological evidence for tissue destruction[1]. A division within the CD8 subset of cytolytic T lymphocytes (CTL) activity and antiviral activity is also indicated by findings with the mutant mouse strain BALB/c H-2^{dm2}, in which the major histocompatibility complex (MHC) L region is deleted. This mutant, in contrast to BALB/c mice, does not generate a significant LCM virus-specific CTL response *in vitro*; this must be taken to mean that in the MHC^d haplotype most LCM virus-specific CTL recognize viral antigens presented by the L^d molecule. Yet, upon adoptive transfer, T lymphocytes of infected BALB/c H-2^{dm2} donors could control virus spread in the spleen of BALB/c recipients, thus suggesting that the antiviral function is not restricted through L^d. To explain this, Lehmann-Grube postulates that an antiviral cytokine blocks viral replication in already infected cells and is released or induced by antiviral effector cells. Because of MHC restriction of antiviral function, the antiviral effector cells must still encounter the few infected cells in a large tissue, but their effect may be potentiated by the protection of neighbouring cells. But whatever the final answer, Lehmann-Grube has drawn attention to a paradox.

Another current research interest of this group is the role of the local LCM virus-induced delayed-type hypersensitivity (DTH) reaction in viral clearance[2]. They have concluded that the DTH reaction to the LCM virus is sequentially mediated by T lymphocytes of the CD8 and the CD4 subsets and that only the first phase is essential for terminating the infection. Recent studies of the antiviral function of gamma-interferon

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(IFN-γ) by infusion of anti IFN-γ antibody showed that in LCM virus infection IFN-γ is not the antiviral cytokine postulated for the effector phase of viral clearance, but is critically involved in the *in-vivo* generation of antiviral effector cells.

Hepatitis A virus – liver pathology caused by infiltrating T lymphocytes

In an interdisciplinary cooperation, the groups of virologist Angelika Vallbracht (Tübingen) and immunologist B. Fleischer (Ulm/Mainz) isolated human T-cell clones from liver biopsies of hepatitis A patients at different stages of the disease[3]. During acute hepatitis, when an elevated level of serum transaminases indicated liver cell destruction, human leucocyte antigen (HLA)-restricted cytolytic clones with the $CD4^-CD8^+$ phenotype and specific for hepatitis A virus-infected fibroblasts were found to be enriched in the liver. In one patient, during a second exacerbation of hepatitis, $CD3^+$ αβ T-cell receptor (TCR)-negative $CD4^-CD8^-$ clones could be isolated from the liver. Vallbracht and Fleischer interpret their findings as evidence for an immunopathogenetic mechanism in hepatitis A.

Corona virus, measles virus and Borna disease – neuropathology elicited by encephalitogenic T lymphocytes of the CD4 subset

V. ter Meulen (Würzburg) and his associates, P.T. Massa, R. Dörries, U.G. Liebert, R. Watanabe, H. Wege and colleagues studied two models of virus-induced acute (AE) and subacute (SAE) encephalomyelitides[4–8]: the experimental infection of the central nervous system of rats with corona virus (strain JHM) and measles virus (strain CAM R/40). In both cases, neonatal or weanling rats succumb to AE, whereas in adult Lewis rats SAE develops with symptoms similar to those known for experimental allergic encephalomyelitis (EAE). EAE is experimentally induced by myelin basic protein (MBP) and can be transferred to naive recipients with MBP-specific T-cell lines of CD4 lineage. Supporting the assumption that the underlying effector mechanism is the same in EAE and virus-induced SAE, MHC class-II-restricted $CD4^+$ T-cell lines specific for rat MBP could be isolated from susceptible Lewis rats during SAE induced by infection with corona or measles virus, whereas in the resistant Brown Norway strain, SAE does not develop and no MBP-specific T-lymphocyte response is generated. As was the case for EAE, MBP-specific lines isolated from SAE rats proved to be encephalitogenic when infused into naive recipients[5]. In contrast, measles-virus-specific $CD4^+$ T-cell lines derived from the same donors were neither encephalitogenic, nor could they be restimulated *in vitro* with rat MBP. The conclusion that measles virus epitopes do not mimic the complete encephalitogenic epitope of rat MBP is supported by the fact that amino acid sequences analogous to the encephalitogenic site in rat MBP are not encoded by the measles virus genome. The mechanism by which the infection aids the generation of self (MBP)-reactive T lymphocytes is not finally resolved. Besides interesting speculations that cannot all be discussed here, evidence is provided by V. ter Meulen and his co-authors for an induction by corona as well as by measles virus of MHC class II glycoprotein expression on astrocytes[6–8], which, in the case of measles virus, can be amplified synergistically by tumor necrosis factor[8]. Such a mechanism can make astrocytes competent to present MBP peptides to MHC class-II-restricted MBP-specific clones.

R. Rott and his group (Giessen) have contributed to viral immunology by recent work on the pathogenesis of Borna disease, a progressive encephalomyelitis of horses and sheep that can also be studied in the Lewis rat model[9,10]. An immunopathogenetic mechanism in Borna disease was already indicated by the finding that manifestations of disease are absent in immunodeficient newborn or athymic rats and after immunosuppression, despite persistent replication of Borna disease virus (BDV) in astrocytes. So far, BDV is only partially characterized. Borna disease could be evoked in persistently infected immunosuppressed recipients by transfer of an MHC class-II-restricted $CD4^+$ T lymphocyte line directed against a BDV-specific protein. Thus, unlike the examples cited above, Borna disease is not a virus-induced autoimmune disease, but is an example of an immunopathology based on expression of viral antigens. This conclusion is supported by the finding that the BDV-specific line is not encephalitogenic in non-infected recipients, while this is a characteristic of the MBP-specific lines in EAE or virus-induced SAE.

Herpes viruses – the immune system succeeds

K.E. Schneweis (Bonn) and his group worked on an experimental model of genital infection with herpes simplex viruses (HSV) types 1 and 2 – members of the herpes virus α subfamily. In immunocompetent mice, virus replicates at the primary site of infection – the epithelial layers of the mucous membranes – and its spreading is restricted to the lumbosacral nerves and their associated ganglia – the site at which viral latency is established. In contrast, after immunosuppression, lymphohematogenous spread of virus results in generalized infection, encephalitis and death[11]. Schneweis concludes that the prevention of lymphohematogenous spread is mainly a function of macrophages and nonrestricted cytolytic cells.

As a model for infection with human cytomegalovirus (human CMV), which is a main cause of mortality in immunocompromised patients, U.H. Koszinowski (Tübingen/Ulm) and his associates studied the infection of mice with murine CMV[12–15]. Strict species specificity within the β herpes viruses precludes the study of human CMV in animal species. Only after immunodepletion by γ-irradiation does the infection result in lethal disease, with interstitial pneumonia, adrenalitis and in particular bone-marrow aplasia being the most critical manifestations. Regimens for an adoptive cytoimmunotherapy of CMV disease were established. Even when the infection is already manifest in vital tissues of the recipient, transferred virus-specific $CD8^+$ T cells can control viral replication and prevent mortality. T lymphocytes of the CD4 subset alone are not antivirally active, nor do they enhance the efficacy of the protecting $CD8^+$ effector cells. To determine whether the CD8 subset can control infection on its own, the course of murine CMV disease was studied in mice depleted of the CD4 subset. Even though viral clearance is delayed in CD4-subset deficient mice, protective $CD8^+$ effector cells are generated and finally restrict persistent viral replication to acinar glan-

dular epithelial cells of the salivary glands.

Murine cytomegalovirus – from the longest viral genome to the shortest antigenic peptide

Another topic that has attracted the interest of Koszinowski and his team for years is the molecular virology and immunology of murine CMV. This work led to the identification of the minimal antigenic sequence in an immunodominant viral protein. Gene expression in herpes viruses is temporally regulated in three phases: the immediate-early, the early and the late phase. Within the 235 kilobase pairs of the DNA genome of murine CMV, immediate early proteins are encoded in a region of approximately 12 kilobase pairs. In the acute immune response of BALB/c mice to local infection, almost half of all CTL in a draining lymph node are directed against antigens specified in this region[16]. The regulatory phosphoprotein pp89, which is encoded by the immediate early gene *iel*, serves as an antigen for polyclonal CTL, and the recombinant vaccinia virus MCMV-iel-VAC protects mice against lethal challenge infection[17]. Deletion mutagenesis of *iel* located the antigenic site within the 595 amino acids of pp89 in a region of 95 amino acids[18]. The nonapeptide YPHFMPTNL (one-letter code), presented by the MHC class I molecule L^d, was identified as the optimal antigenic peptide for the pp89-specific CTL clone IE1 (CD8, TCR α/Vβ6). With the sequence HFMPT, this nonapeptide contains an antigenic motif predicted by J.B. Rothbard (London). The demonstration of a direct antigenicity of the pentapeptide HFMPT has added a new aspect to the current understanding of the MHC molecule–peptide–TCR interaction[19].

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AIDS research

Johanna L'age-Stehr

Research on the acquired immune deficiency syndrome (AIDS) had a somewhat delayed start in West Germany, similar to the time lag of about two years for the start of the German AIDS epidemic compared with the situation in the United States[1]. Initial studies focused mainly on clinical disease manifestation, epidemiological studies, descriptions of immune dysfunctions in AIDS patients and the development of methods for the diagnosis of AIDS-related disease. More recently, detailed studies have begun on HIV-related immune dysfunctions, pathogenetic mechanisms, biology of the virus–host relationship and therapeutic vaccination approaches.

Immune response and dysfunctions in HIV-infection and AIDS

The group of G. Riethmüller, P. Rieber, H.W.L. Ziegler-Heitbrock and colleagues (University Munich) was among the first to study the changes of the cellular components of the immune system in AIDS. They showed an increase of $CD8^+/CD32^+$ (Leu 7^+) T cells in HIV-infected hemophiliacs[2]. Most of these T cells are activated[3]. They also showed depletion of $CD4^+$ monocytes and interpreted this as the result of an autocytotoxic reaction of T lymphocytes[4].

W.L. Gross, I. Kekow and coworkers (University Kiel) showed a profound B-cell dysfunction in HIV-infection. Using T-cell-independent B-cell mitogens and polyclonal B-cell activators, they found that abnormal B-cell proliferation and differentiation responses correlated with the progress of disease. Interleukin 2 (IL-2) can partially restore the impaired differentiation response[5–8].

P. Racz, K. Tenner-Racz and colleagues (Tropeninstitut, Hamburg) together with collaborators in France (J.C. Gluckman), England (G. Janossy) and USA (M. Popovic) were among the first to describe changes in architecture, structure and cellular composition of lymph nodes in different stages of HIV infection. They identified the germinal centers and especially follicular dendritic cells as important reservoirs for HIV and as possible sites for selection of HIV strains with altered target cell tropism[9–11].

Sethi and colleagues (Progen, Heidelberg) have found major histocompatibility complex (MHC)-restricted HIV-specific cytotoxic T

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cells in AIDS patients, particularly in the cerebrospinal fluid, that specifically destroy HIV-infected autologous macrophages[12]. They have proposed this phenomenon as one of the possible pathogenic mechanisms for brain damage in HIV-infection.

The role of CD8+ lymphocytes during HIV infection is being studied by B. Koszinowski's group (University Ulm); they are also trying to define the viral gene products that are recognized by cytolytic T cells.

Within a multicenter prospective cohort study, begun in 1984 by M.A. Koch (Federal Health Office, Berlin), the quality and quantity of circulating immune complexes were examined by J.R. Kalden and V. Krapf (University Erlangen) in follow-up studies of several hundred HIV infected homosexuals. A significant increase in the amount of circulating immune complexes is correlated with the duration of the HIV-carrier state and the development of HIV-related diseases.

Biology and molecular characterization of HIV

Based on their extensive experience in analyzing the fine structure of retroviruses, H. Gelderblom, M. Özel and G. Pauli (Robert Koch Institut, Berlin) showed as early as 1984/1985, that by morphological comparison with animal retroviruses, HIV can be classified as a lentivirus. Their electron microscope studies led to the elucidation of the structure and architecture of the virion and became the basis for a detailed model[13]. The virus envelope is studded with 72 glycoprotein knobs (trimers of gp120) which are shed during morphological maturation and aging of the virion[14,15]. Host cell components such as MHC class I and II antigens can be identified in the viral envelope[15]: these findings may be significant in the pathogenic mechanisms of AIDS. In phases of massive viral replication, shed gp120 attaches to membrane CD4 receptors; this can direct HIV antibody-dependent cellular cytotoxicity to CD4+ cells, and lead to depletion and functional impairment of uninfected CD4+ cells.

H. Rübsamen-Waigmann and coworkers (G. Speyer-Haus, Frankfurt) were among the first in Germany to succeed in isolating and cloning different HIV strains and subtypes from cerebrospinal fluid and peripheral blood of patients, and to demonstrate different cellular tropism for these isolates[17].

K. Mölling and colleagues (MPI, Berlin) analyzed the replication of HIV and characterized the viral enzymes, reverse transcriptase, RNAseH, protease and endonuclease and studied mutagenesis as well as inhibitors of viral replication[18–20] mainly as the basis for future therapeutic approaches.

By expression cloning of structural HIV protein genes and open reading frames (ORFs) for regulatory polypeptides, B. Fleckenstein and G. Jahn (University Würzburg) in collaboration with W.A. Haseltine (Boston, USA) aim to improve diagnostic procedures, and to study the synthesis of structural proteins[21–23].

HIV infection, target cells and pathogenic mechanisms

HIV displays a selective tropism for CD4+ cells. Besides being found in T cells, persistent productive HIV-1 and HIV-2 infections have been found mainly in macrophages. Other antigen-presenting cells can also become persistently infected, as was shown for follicular dendritic cells by Tenner-Racz *et al.*[10] and for Langerhans cells by R. Kunze and colleagues (Robert Koch-Institut, Berlin) and L. Braaten (Oslo)[24].

CD4 was shown to be the receptor for HIV in human brain by I. Funke, G. Riethmüller *et al.* by using different epitope-specific monoclonal antibodies and CD4 cDNA. Two forms of mRNA were found in the human cerebellum[25].

W. Mellert, V. Erfle and colleagues (GBF, Munich) in collaboration with M. Popovic (USA) aim to clarify the role of glial cells as an HIV reservoir in the central nervous system. Several glioma and astrocytoma cells were converted to chronically HIV bearing lines expressing several structural and regulatory HIV proteins. The infection with HIV has a remarkable influence on growth properties of glial cells[26].

V. ter Meulen and colleages (University Würzburg) showed that HIV replication in the CNS is reflected by synthesis of intrathecally produced viral antibodies to HIV structural proteins.

Using double labelling fluorescein activated cell sorter (FACS)-analysis, E. Wecker, T. Kerkau and co-workers (University Würzburg) found that during the first five to six days post infection, expression of HLA-class I antigens is down-regulated in CD4+ HeLa cells and peripheral mononuclear CD4+ T cells, while mRNA is not. This observation is suggested as a possible explanation for the failure of cellular immune response in HIV-infected patients.

A. Ganser, D. Hoelzer and colleagues (University Frankfurt) found that hematopoietic progenitor cells were decreased in bone marrow and peripheral blood of patients with HIV-infection[28,29] which they propose is due to the inversion of CD4/CD8 lymphocytic subpopulation ratio, while their response to hemopoietic stimulating factors is normal.

The group of P.H. Hofschneider (MPI, Martinsried) has investigated the cellular origin of AIDS-Kaposi's sarcoma (KS). Cultivated KS cells express endothelial cell markers as do their counterparts in the tumor, indicating that these cells have an endothelial origin[30,31]. In culture, the growth of KS cells is contact-inhibited and they become senescent after 30–40 passages; overexpression of common oncogenes is not observed; the growth of KS cells in culture is strongly dependent on platelet-derived growth factor (PDGF) as a major mitogen. KS cells secrete growth factors which can act in an autocrine fashion. The following tumor model is postulated: endothelial cells and macrophages present in the tumor stimulate KS cells with PDGF and additionally, KS cells promote their own growth in an autocrine manner.

The possible role of cytokines (e.g. interleukin 4) in the pathogenesis of AIDS is being studied by P.H. Krammer (DKFZ, Heidelberg) and colleagues. A depression of IL-4 secretion might lead to a depression of the helper T cell pool, since IL-4 acts as an autocrine growth factor for helper T cells. They have evidence of a deregulation of the IL-4 gene after HIV-infection.

Vaccine development, therapeutic approaches and animal models

The development of HIV vaccines is being examined here mainly by three groups: G. Hunsmann (Primate Center, Göttingen), H. Wolf (University Munich) and R. Kurth (Paul Ehrlich Institut, Frankfurt). H. Wolf, with S. Modrow, M. Motz and colleagues work on identifying the

immunorelevant epitopes of HIV using computer-assisted analysis. They identified the envelope protein with their antigenic epitopes and constant and variable regions within the amino acid sequence of various HIV-isolates and studied their role in the humoral and cellular immune response using synthetic oligopeptides[32]. The macaque model for HIV vaccine and treatment trials is being studied by G. Hunsmann and co-workers (Göttingen). Rhesus monkeys vaccinated with purified glycoproteins of simian immunodeficiency virus (SIV) develop high neutralizing antibody titers. Experiments to challenge these animals with pre-titrated SIV are planned[33]. R. Kurth (Paul Ehrlich-Institut, Frankfurt) and colleagues have isolated, cloned and sequenced SIV from African green monkeys[34,35].

G. Köhler (MPI, Freiburg) is trying to clone anti-mouse CD4 antibody encoding genes to analyse transgenic mice carrying these genes for lack of CD4+ cells and their immune status. F. Emmrich's group (University Erlangen) have produced an anti-human CD4 monoclonal antibody that efficiently blocks both the binding of gp120 to CD4, and HIV infection *in vitro*. Using this antibody, a series of high affinity combining site associated anti-idiotypic (anti-id) antibodies were generated in syngeneic mice, but to date no internal image idiotype of CD4 that recognizes gp120 and blocks HIV infection has been obtained. G. Riethmüller's group is using a similar approach, but have not been successful in finding internal image antibodies either. A screening system for antiviral compounds has been developed by H. Rübsamen-Waigmann and co-workers. With this system, pentosan-polysulfate (HOE/BAY 946) was shown to inhibit HIV-1 and HIV-2 strains with comparable efficiency in cell culture, and with very low toxicity. Furthermore, the substance was found to inhibit virus synthesis, and may have potential as a therapeutic agent in early stages of HIV infection to prevent development of AIDS[36].

It was proposed by W. Dröge's group (DKFZ, Heidelberg) that reduced plasma cysteine (thiol) levels in HIV-infected patients are responsible for reduced lymphocyte and macrophage function[37] and they suggest *N*-acetyl-cysteine for treatment of HIV-1 infected patients.

The focus of the research of M. Pawlita, H. zur Hausen and their colleagues (DKFZ, Heidelberg) is on inhibition of HIV expression and replication by using the molecular genetic principle of cloning and inducing 'anti sense' RNA for viral genes in HIV-infected cells.

K. Helm's group (University Munich) are working on the approach of inhibiting assembly of HIV by blocking the virus coded protease.

Federal government research program on AIDS

In October 1983 the Federal Minister for Research and Technology (BMFT) announced that special funding had been granted for a research program on AIDS to be administered by the Federal Health Office (BGA). The BMFT Research Program on AIDS provides funds to universities and other research establishments including industrial laboratories. Grant applications undergo a strict peer review process including evaluation by overseas referees. To date more than 80 laboratories or research establishments are participating in the Research Program on AIDS, including experts in the fields of molecular genetics, immunology, virology, biochemistry, clinical medicine and epidemiology.

In June 1987, a special grant program for multi-disciplinary projects was initiated to improve clinical research and to strengthen the collaboration of basic and clinical research in the Federal Republic of Germany.

Perhaps more than any other area, development of effective AIDS research is dependent on international collaboration and cooperative efforts. In 1985, AIDS research was officially included in the existing Bilateral Agreement between the USA National Institutes of Health (NIH) and the BMFT on Cooperation in Biomedical Research (coordinators for AIDS research within the agreement for the USA side: A. Fauci and for the German side: J. L'age-Stehr). The initiatives for cooperation in various research projects should derive primarily from the individual scientists who make contact through established scientific channels; the bilateral agreement aims at facilitating and intensifying early information on new developments and research priorities and to help to ensure the optimum use of the available resources. In 1988 similar agreements had been made between the BMFT and the Medical Research Council (MRC) in Great Britain, the INSERM in France and the Ministry for Health and Welfare in Japan. Furthermore, the German AIDS research activities are embedded in the concerted action of the European Community for Coordination and Support of AIDS Research.

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