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.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Veterinary Immunology and Immunopathology, 4 (1983) 237—277
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

THE IMMUNE RESPONSE TO VIRUS INFECTIONS

D.E. ONIONS

Department of Veterinary Pathology, University of Glasgow Veterinary School, Bearsden Road, Bearsden, Glasgow G61 1QH, Great Britain

TABLE OF CONTENTS

1. INTRODUCTION

2. VIRUS STRUCTURE AND ANTIGENS

2.1 Structural types
2.2 Surface antigens
2.3 Antigenic diversity of surface antigens
2.4 Internal antigens

3. VIRUS HOST-CELL INTERACTIONS

3.1 Lytic infections by non-enveloped viruses
3.2 Enveloped virus infections
3.3 Latent virus infections
3.4 Oncogenic transformation

4. VIRUS NEUTRALISATION

4.1 Mechanism of neutralisation
4.2 The persistent fraction

5. THE ROLE OF COMPLEMENT IN ANTI-VIRAL IMMUNITY

5.1 Neutralisation by antibody and complement
5.2 Non-immune activation of the complement system
5.3 Lysis of cells by antibody and complement
5.4 Immunopathology associated with the humoral response

6. ANTIBODY DEPENDENT CELL MEDIATED CYTOTOXICITY

0165-2427/83/$03.00 © 1983 Elsevier Science Publishers B.V.

7. CELL MEDIATED CYTOTOXICITY

7.1 Natural killer cells
7.2 The N.K. cell
7.3 Genetic control of N.K. expression
7.4 Target cell specificity of N.K. activity
7.5 Interferon and N.K. activity

8. CYTOTOXIC T-CELL RESPONSE

8.1 MHC restriction of cytotoxicity
8.2 The development of the cytotoxic response
8.3 Antigens recognised by cytotoxic T-cells

9. CONCLUSION

10. REFERENCES

1. INTRODUCTION

Since the immune system has evolved primarily as a defense against infectious agents it is not surprising to find that the full repertoire of this system can be evoked by viruses. Although there is considerable interaction between the elements of the immune response, it is convenient to distinguish between humoral and cell-mediated components. The most rapidly developing humoral and cell-mediated responses are represented by primordal arms of the immune system, the alternative pathway of complement and natural killer cells. A slightly later developing response, T-cell mediated cytotoxicity, acts to eliminate virus infected cells and it is often the major determinent in recovery from primary infections by enveloped viruses. The antibody response can also be an important element in recovery from primary infections but in addition the neutralising antibody response forms the major barrier to reinfection by viruses.

2. VIRUS STRUCTURE AND ANTIGENS

2.1 Structural types

Although there are approximately 20 well defined groups of animal viruses there are only a limited number of structural types. The smaller viruses exhibit the simplest structure in which nucleic acid either RNA or DNA is surrounded by a protein coat called the capsid. In most of the DNA

viruses, and in the smaller RNA viruses, the capsids are icosahedral in shape and therefore display cubic symmetry. The capsids are composed of repeated structural units or capsomeres which, in turn consist of a few viral coded proteins.

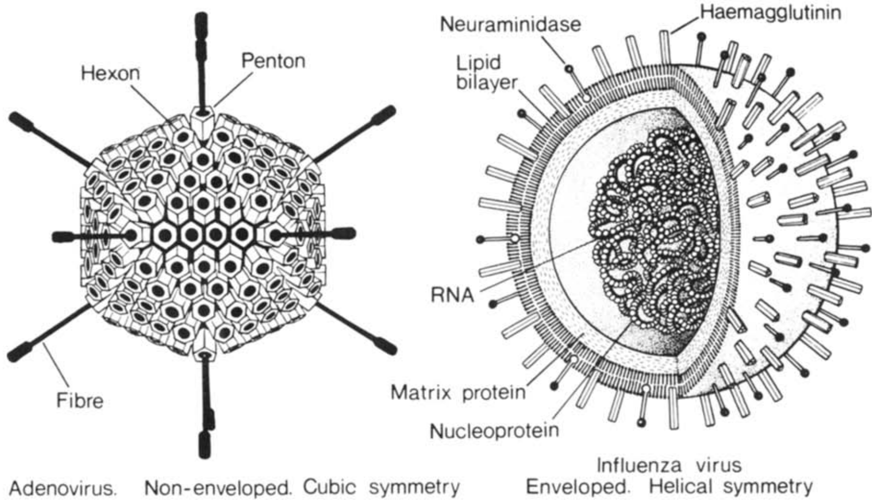
The larger RNA viruses have a different arrangement of the capsid, the capsomeres being wound around the nucleic acid to form a long helical structure known as the nucleocapsid (Fig. 1). The nucleocapsids of the viruses with helical symmetry and some viruses with cubic symmetry, like the togaviruses and herpes viruses, are surrounded by an envelope derived from a modified cell membrane. This is acquired as the virions (virus particles) are liberated through the plasma membrane, or in the case of the herpes viruses as they pass through the nuclear membrane. The phospholipid, glycolipid and cholesterol components of the envelope are derived from the host cell but relatively little (0.5 to 1.0%) of host protein remains in the envelope. Amongst the remaining host proteins actin has been identified in some virus envelopes and histocompatibility antigens have been demonstrated in rhabdoviruses and retroviruses (Griffin and Compans, 1979; Bubbers and Lilly, 1977). In place of these host proteins, viral coded proteins become substituted into the envelope. In most of the enveloped viruses, except the togaviruses, the envelope overlies a matrix protein which plays an important role in the assembly and release of the virions. In addition to the strutural proteins some viruses carry enzymes necessary for their replication.

Although most viruses possess capsids with helical or cubic symmetry some have a more complex structure. Retroviruses have a helical nucleocapsid surrounded by an outer icosahedral capsid whilst pox viruses have an even more elaborate substructure of membranes and enzymes.

2.2 Surface antigens

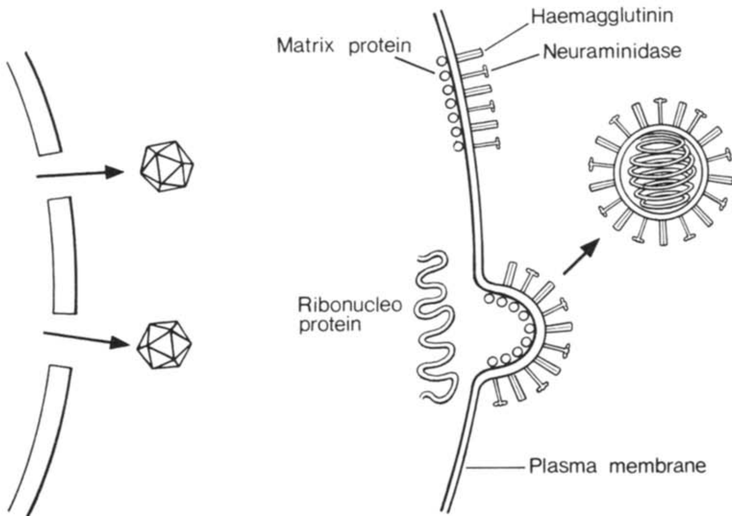
Virus infections present the immune system with a range of proteins which are antigenic. The biological importance of a viral antigen is, however, largely dependent on its location and on the function of the antigen in viral replication. Neutralising antibody, that is antibody that renders a virus non-infectious, is directed towards antigens on the surface of virions. In the case of enveloped viruses these are the glycoprotein spikes of the virus. Exceptions occur however. In bovine leukaemia virus infections, for instance, antibody is produced to glycosolated determinants, whereas immunisation with inactivated virus results in antibody to protein components (Portelle et al., 1981). Cytotoxic T-cells

VIRUS STRUCTURE AND RELEASE



Adenovirus. Non-enveloped. Cubic symmetry

Influenza virus
Enveloped. Helical symmetry



Lytic release of an unenveloped virus, no virus antigen in plasma membrane

Release of Influenza virus by budding

Figure 1.

also recognise carbohydrate (Ertl and Ada, 1981) and it has been suggested that genes in the major histocompatibility complex code for glycosyl transferases, which attach specific oligosaccharides to viral proteins to render them antigenic (Blanden et al., 1976). The glycoproteins of the viral envelope are also expressed on the cell surface but in addition antigens not normally accessible at the surface of the virion can be found in the cell membrane. The matrix protein of influenza virus has been reported to be expressed at low levels on the cell surface (Hackett et al., 1980) and in feline leukaemia virus infections a novel antigen consisting of the glycosalated, internal antigen, precursor protein is expressed on the plasma membrane (Neil et al., 1980).

The surface proteins of non-enveloped viruses are not glycosalated but, as in enveloped viruses they are targets of neutralising antibody. In addition, the surface proteins of both enveloped and non-enveloped viruses may bind to erythrocytes and haemagglutinate them. Antibody specific for the haemagglutinins of viruses will inhibit their haemagglutinating activity and this reaction provides a convenient method for measuring anti-virus antibody.

The maintenance of repeated epitopes, in a regular fashion, on the surface of viruses might be expected to indicate that these structures would function as thymus independent antigens. Experimental investigation of the humoral response of athymic nude mice (nu/nu) to virus infections has suggested, however, that the response to these antigens is either completely thymus dependent or requires a thymic function for switching from an IgM to an IgG response (Burns, 1975).

2.3. Antigenic Diversity of Surface Antigens

Antigenic diversity of surface antigens within a virus type is frequently encountered. When the host humoral response discriminates between these antigenic types they are referred to as serotypes. An animal immune to one serotype is usually susceptible to infection by another serotype of the same virus. This antigenic diversity of viruses, as detected in neutralising antibody assays, is not necessarily a reflection of their structural complexity. Foot and mouth disease virus has a simple structure yet it has seven major serotypes whilst, on the other hand, herpes viruses have a complex structure and yet they are usually monotypic. Antigenic variants of viruses will arise through mutation or genetic recombination, and whether or not these new variants persist will be dependent on the selection pressure. In virus infections at mucosal

surfaces, as in foot and mouth disease, a balance between virus replication and the immune response can occur that favours the selection of new antigenic variants. In contrast, in herpes virus infections the virus can evade the immune response, both by direct cell to cell spread of infection through cell fusion or by remaining as a latent infection. Consequently there is less likely to be strong selection pressure for new antigenic variants within these viruses.

In viruses with segmented genomes, like influenza virus, a special form of recombination known as genetic reassortment is possible. When two different influenza viruses infect the same cell, the eight segements of the genome of each virus can be replicated and packaged into new combinations in the progeny virions. These new genetic combinations lead to radical changes in antigenicity (antigenic shifts) of the haemagglutinin spikes which contrast with the less extensive antigenic change (antigenic drift) associated with mutation.

2.4 Internal antigens

Antibody is produced to internal antigens of viruses but these are not exposed in intact virions and therefore do not contain targets for neutralisation. Consequently there is less selection pressure for antigenic variants and often internal antigens are common to members of different serotypes. This property is important when choosing a serological test. When influenza-A viruses are examined by the haemagglutination inhibition test different serotypes will be detected since only the antigenicity of the variable haemagglutinin is being examined. However if the complement fixation test is used all the virus antigens will react, including the group specific ribonucleoprotein antigens.

Although internal antigens are not biologically important in neutralisation, they can be important in the immunopathology of some virus diseases. In the pathogenesis of immune complex disease the principal feature is the deposition of antigen-antibody-compexes which include an important, and sometimes predominant, component of internal antigens.

3. VIRUS HOST-CELL INTERACTIONS

The nature of the immune response to viruses is governed in part by the relationship of the virus with the host cell. In examining these relationships it is convenient to consider the non-enveloped viruses and the enveloped viruses separately.

3.1 Lytic infections by non-enveloped viruses

Viruses are obligate intracellular parasites dependent on host cell machinery for their replication. In order to enter a cell a virus must possess sites on its surface that are complementary to receptors on the cell surface. Once inside the cell the virus sheds it protein coat releasing the nucleic acid. The genome of the virus now directs the synthesis of a variety of proteins which are required for replication of viral nucleic acid and for incorporation into new structural components of the virions. When sufficient concentrations of proteins and nucleic acids have been made they are assembled into progeny virus particles.

After assembly the progeny virions are released from the cell over a period which varies with the virus type. In general, the non-enveloped viruses are released rapidly, a process which results in lysis and death of the cell. Furthermore as viral coded proteins are not found in the cell membrane the immune response is not directed against infected cells but is predominantly a humoral response against extracellular viruses. (Fig. 1).

3.2 Enveloped virus infections

Entry of enveloped virus is again dependent on the presence of cell receptors on the virus and these take the form of glycoprotein spikes projecting from the surface of the envelope. Release of enveloped viruses occurs through a budding process at the cell membrane and as in non-enveloped viruses this may result in lysis and rapid death of the cell. However, for some enveloped viruses like paramyxoviruses, release of the virus may take place over several days without much evidence of cell destruction. This is seen in extreme form in the retroviruses, in which infected cells may continuously release virus without any effect on cellular function. For the enveloped viruses, virus coded proteins are present in the cell membrane before the release of virions. Consequently the immune system, can recognise and eliminate virus infected cells before the release and spread of virus occurs. (Fig. 1).

3.3 Latent virus infections

A property of the members of certain virus groups particularly those in the herpesvirus, retrovirus, and paramyxovirus groups is their ability to remain as latent infections. In this state the virus is represented in the host cell by its genome without the production of complete virions. In herpes virus infections the latent state is usually maintained in neurones or lymphoid tissue whilst in feline leukaemia virus infections (a retrovirus) latency is maintained in the bone marrow (Rojko et al., 1981). Reactivation from the latent state with the production of new virus particles can occur and in herpes virus infections this may also be associated with the recrudescence of clinical symptons. Amongst the agents associated with reactivation from latency are a range of immunosuppressive products. In the case of feline leukaemia virus (FeLV) the latent state appears to be associated with an active cellular and humoral immune response directed against the expression of viral antigens on the cell surface (Rojko et al., 1981). What is not clear is whether spontaneous reactivation from latency is occurring frequently, with the immune response acting to eliminate cells expressing viral antigens or, whether the immune response is directly responsible for maintaining the latent state by modulating virus expression.

3.4 Oncogenic transformation

Oncogenic transformation may be produced by a number of DNA virus groups and by one RNA virus group, the retroviruses. Transformation of cells by retroviruses is often associated with continued release of virus from the tumour, but in transformation induced by DNA viruses production of new virions does not occur. In both cases, however, expression of novel, virus coded and virus induced antigens can occur on the neoplastic cell surface. These tumour associated antigens can function as targets for the immune reponse in the elimination of neoplastic cells. Important veterinary examples are the FOCMA antigen expressed on FeLV transformed cells (Essex et al., 1971) and the MATSA antigen found on Marek's disease tumour cells (Powell et al., 1974; Witter et al., 1975).

4. VIRUS NEUTRALISATION

The choice of test used to measure antibody responses to viruses is often based on pragmatic criteria. Complement fixation and enzyme linked

immunosorbent assays provide easily automated screening tests for examining large numbers of samples in epidemiological surveys. When whole virus is used as antigen in these tests, however, subgroup specificity is often obscured by the group specific reactions. In those viruses that haemagglutinate, the haemagglutination inhibition test provides a quantitative assay that is inexpensive, quick to perform, and furthermore often subgroup specific.

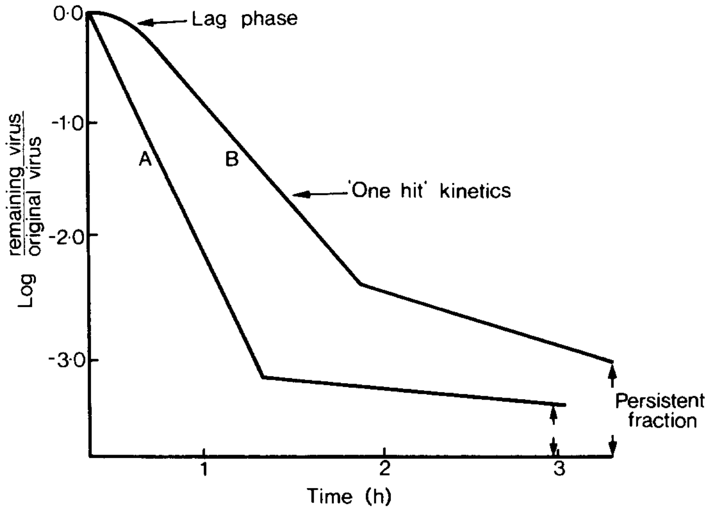
In the range of available serological tests virus neutralisation occupies a special position since, by definition, it detects antibody that results in a loss of virus infectivity. Virus neutralisation assays therefore provide a means of assessing the protective humoral response in vitro. The assays are conducted in two stages; a virus of known titre is first incubated with heat inactivated serum under test and after an interval of 30 minutes to 1 hour the residual infectivity is measured in an indicator system. The sensitivity of the assay is largely governed by the sensitivity of the indicator system which is usually a permissive cell culture. Most neutralisation assays are conducted with a constant amount of virus and a variable (titred) serum concentration. In these assays antibody is usually in excess and, under these conditions, the percentage of surviving virus after neutralisation is independent of the initial quantity of virus employed.

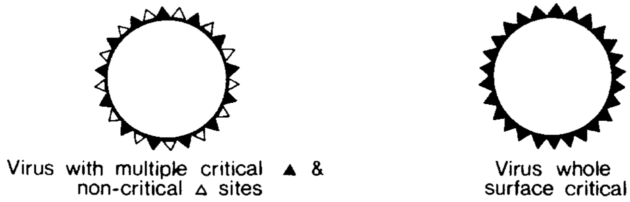
4.1 Mechanism of neutralisation

In order to replicate a virus particle must bind to a target cell, enter it and finally uncoat to liberate its nucleic acid genome. Neutralising antibody has been shown to interfere with each of these functions.

When the surviving fraction of virus is examined at different times in the nuetralisation reaction, curves illustrated in figure 2 are generated. In curve A an immediate decrease in the surviving fraction is observed with the same proportion of virus being neutralised in a given time interval. This pattern of 'one hit' kinetics has been taken to indicate that only a single antibody molecule is required to neutralise each virus particle. However when the same neutralisation reactions are conducted at low antibody concentrations, or low temperature, a change in the curve occurs with a lag in the onset of rapid neutralisation. This lag phase is consistent with a model proposed by Meynell and Meynell (1970) in which several antibody molecules are required to attach to the virus in order to effect neutralisation. Once a critical number of antibody

VIRUS NEUTRALISATION





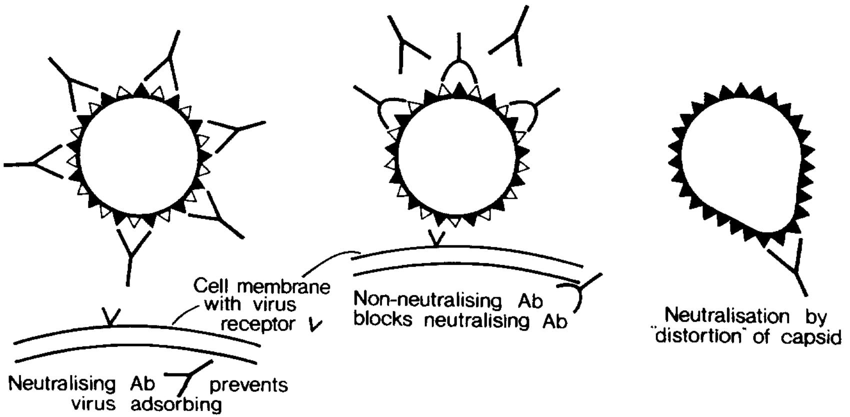


Figure 2.

molecules have combined with the virion surface, the neutralisation of the virion-antibody complex is dependent on the addition of one final antibody molecule. This is indicated by the transition of the neutralisation curve to a linear pattern. (Fig. 2B).

Two main lines of evidence support the contention that for most viruses neutralisation is dependent on binding several antibody molecules. Firstly infectious antigen antibody complexes occur which can be neutralised by the addition of anti-immunoglobulin or by the addition of the early acting components of complement (Oldstone 1975). Secondly antibodies directed at different determinants on the virion surface act synergistically in neutralisation.

Not all the determinants on the surface of a virion are necessarily involved in neutralisation and one can make the distinction between critical and non-critical sites for neutralisation (Fig. 2). In influenza virus the envelope contains multiple units of haemagglutinin and neuraminidase antigens (Fig. 1). The critical sites for neutralisation in this virus are the haemagglutinin spikes whilst the neuraminidase spikes play little or no role in neutraliation. The haemagglutinins of influenza virus are required for binding and entry into the host cell and in a simple model one can envisage that a majority of these receptor molecules must be coated to strearically inhibit the virus from binding to its target (Fig. 2). However the mechanism of neutralisation may be more complex as some evidence suggests that neutralised influenza virus adsorbs to, and uncoats in suitable host cells but transcription of the viral genome does not occur (Dimmock and Possee, 1981). It has been suggested that virus-antibody complexes may activate cellular nucleases and this may account for the neutralisation of virus once it has entered a cell (Mandel, 1967).

In adenoviruses the definition of critical sites is more complex. The capsid consists of 240 hexon capsomeres and 12 penton base capsomeres arranged at the vertices of the icosahedron. Projecting from the penton base is a fibre; the complex of the fibre with the penton base being termed a penton (Fig. 1). Hexons possess distinct sites with group specific and type specific determinants, whereas type specific determinants predominate on the fibre (Norrby, 1971; Pettersson, 1971). Neutralising antibody is directed largely at the hexons, whilst anti-penton serum has only weak neutralising activity and anti-fibre serum hardly any at all (Kjellen and Pereira, 1968). Although anti-penton serum has low neutralising activity, it considerably enhances the neutralising effect of anti-hexon antibody (Hierholzer and Dowdle, 1970) and furthermore anti-penton serum alone can have a significant neutralising capacity on the

addition of antiglobulin (Norrby and Wadell, 1972). This suggests that critical sites are not confined to hexons alone and that maximal neutralisation reactions involve antibody reactions at more than one site. A possible exception to multiple site neutralisation occurs in the Picornaviridae. For polio-virus it has been found that the combination of a single antibody molecule is associated with a change in the isoelectric point of the virus and this has been taken to indicate that a distortion in the capsid structure precedes neutralisation (Mandel, 1976). (Fig. 2).

4.2. The persistent fraction

The rapid phase of neutralisation obeying first order kinetics is followed by a slower phase. This levelling off of virus neutralisation can be pronounced leaving a persistent fraction (Fig. 2). Persistent fractions are more frequently observed with low avidity antibody, occurring early after infection or immunisation. This antibody disassociates from the virus-antibody complexes on dilution leading to reactivation of the virus (Lafferty, 1963). It has been suggested that persistent fractions can also arise when the virus forms aggregates in which some virions are unavailable to the neutralising antibody. However persistent fractions have been observed when monodispersed virus and high avidity antibody have been used in the neutralisation reaction. Under these conditions the addition of further immunoglobulin to the persistent fraction does not result in additional neutralisation but when a Fab fragment is employed further neutralisation occurs (Ashe et al., 1969). A reasonable interpretation is that the smaller Fab fragment is able to reach sites on the virion inaccessible to the larger intact immunoglobulin. This stearic inhibition of neutralising antibody could occur through the binding of antibody to non-critical sites leaving the critical sites available to interact with the host cell (Fig. 2).

5. THE ROLE OF COMPLEMENT IN ANTIVIRAL IMMUNITY

An increasing body of evidence indicates the importance of complement in the antiviral response. Complement can play a role in neutralising free virus and in eliminating virus infected cells. These activities are initiated in the following ways:

(i) Immune activation of the complement pathway leading to virus neutralisation.

(ii) Lysis of virus infected target cells by antibody and complement.

(iii) Non-immune activation of the alternative or classical pathways of complement by virions or virus infected cells.

5.1 Neutralisation by antibody and complement

Enveloped viruses should in theory be susceptible to damage through activation of the terminal phospholipase of the complement pathway. The addition of purified components of the complement system in sequence have been shown to enhance the neutralisation of equine arteritis virus-antibody complexes (Radwan and Crawford, 1974). Similarly the addition of complement to retrovirus-antibody complexes is associated with a loss of structural integrity of the viral envelope and a consequent loss of infectivity (Oroszlan and Gilden, 1970; Oldstone, 1975). In avian infectious bronchitis virus, a coronavirus, Berry and Almeida (1968) demonstrated punctate lesions in the envelope associated with lytic complement damage. However, they also found that neutralisation could occur without these lesions being evident, indicating that virolysis was not an absolute requirement for ablating infectivity.

These indications that virolysis is not necessarily a prerequisite for neutralisation of enveloped viruses have been confirmed by the addition of the early components of the complement pathway to herpes virus-antibody complexes (Daniels et al., 1970). During the sequential addition of C1, C4, C2 and C3, significant neutralisation was first detected upon the addition of C4 and increased as C2 and then C3 were added. Neutralisation by these early components probably occurs through masking of cell receptors on the virion surface although aggregation of viral particles by C3 has been suggested as a possible mechanism (Oldstone et al., 1974). The binding of C3b to the virion may also play an important role in the removal of the immune complexes by the phagocytic cell system whilst the anaphylatoxin C3a, generated during the cleavage of C3, promotes an inflammatory response that appears to be important in limiting the cell to cell spread of herpesviruses and paramyxoviruses.

5.2 Non-immune activation of the complement system

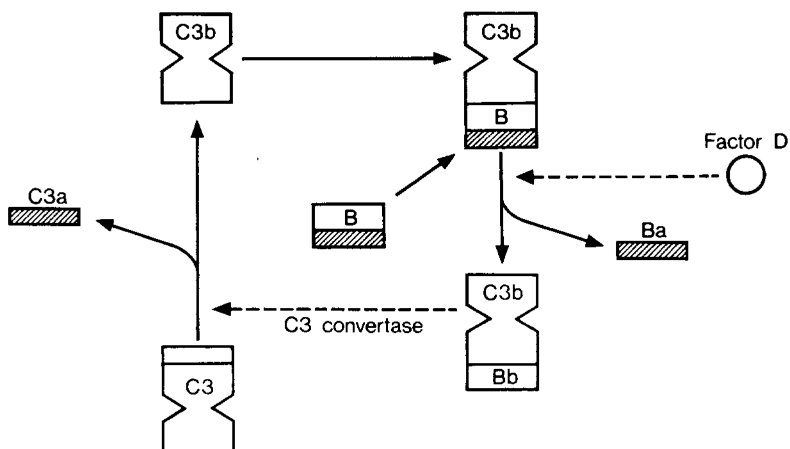
A striking feature of the alternative pathway of complement is that it can be activated in the absence of immunoglobulins. This may reflect the posited evolutionary origin of the alternative pathway as an amplification system for inflammatory reactions which, through later gene duplications,

has formed the classical and terminal pathways (Lachman and Hobart, 1979). The essential featues of the alternative pathway are illustrated in Figure 3. This pathway is a positive feedback cycle resulting in the cleavage of C3. (Müller-Eberhard and Götze, 1972). Starting with the cleavage product C3b, this combines with factor B to form a complex with little enzymatic activity. The complex C3bB is, however, susceptible to cleavage by factor D which is present in serum in an active form. The product of this enzymatic splitting, C3bBb, is a powerful C3 convertase which initiates another round of the cycle by cleaving C3 to C3b and C3a. Once formed, C3b can transfer from the fluid phase to the surface of a biological particle. The binding site on C3b required for this transfer has a finite life beyond which the molecule will remain in the fluid phase. This feature acts as a local control limiting the effects of activation to a specific target. Regulation of the level of activation is dependent on control of the C3bBb concentration. This can occur in two ways; firstly, the C3bBb complex can spontaneously decay to release C3b and an inactive Bbi. Secondly, factor H competes with factor B for binding to C3b. Once bound to factor H, C3b is inactivated by factor I (Whaley and Ruddy, 1976).

It has been suggested that the alternative pathway is constantly 'ticking over' resulting in the random deposition of complement (Lachmann and Nicol, 1973). Whether or not a continued deposition of C3b occurs on a paticular surface will be dependent on the extent that the bound C3b is available to factor H (Pangburn et al., 1980). This hypothesis is illustrated in figure 3 in which the C3b binding site on "activating surfaces" masks or inactivates the factor H binding site and allows the formation of C3bBb. Activation of the alternative pathway in the absence of immunoglobulin by virus infected (Sissons et al., 1980) and virus transformed cells (Budzko et al., 1976; McConnell et al., 1978) has been reported recently. Although activation of complement occurred without antibody, lysis of a range of human cells infected with enveloped viruses required both an intact alternative pathway and an anti-viral IgG. Even in the absence of lysis C3b coated cells could become susceptible to damage by phagocytic cells (Fig. 4C).

Non-immune activation of the complement pathway is also produced by virus particles. Retroviruses can be lysed by fresh human serum but not by serum from a number of other species (Welsh et al., 1975). This process occurs through activation of the classical pathway although a functional alternative pathway greatly increases the virolysis obtained (Cooper

THE ALTERNATIVE PATHWAY OF COMPLEMENT



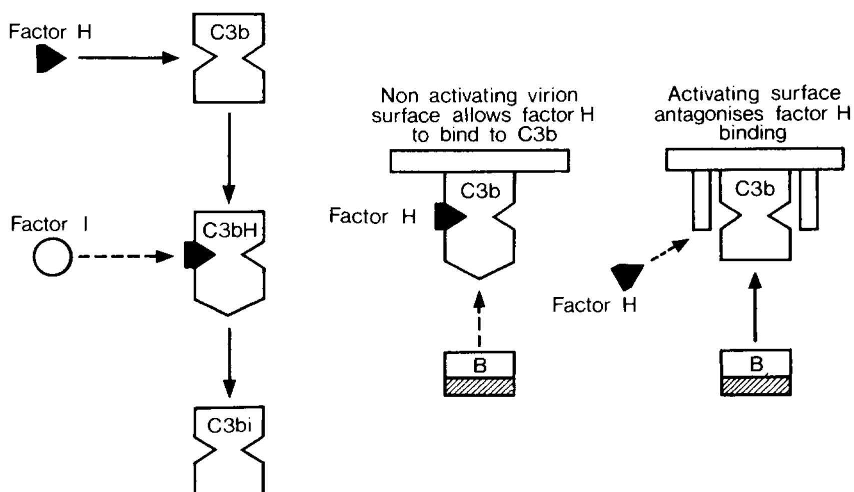


Figure 3.

et al., 1976). The activator for Cl on the virion surface appears to be P15(E) which is analagous to the fusion proteins of other viruses (Bartholomew et al., 1978). Welsh et al. (1975) and Cooper et al. (1976) have suggested that the capacity of serum to lyse retroviruses without antibody may be related to the absence of retrovirus viraemias in those species. In this regard it is of interest that plasma viraemias of retroviruses occur in cats and that the serum of this species does not lyse retroviruses (N. Gorman pers. comm.).

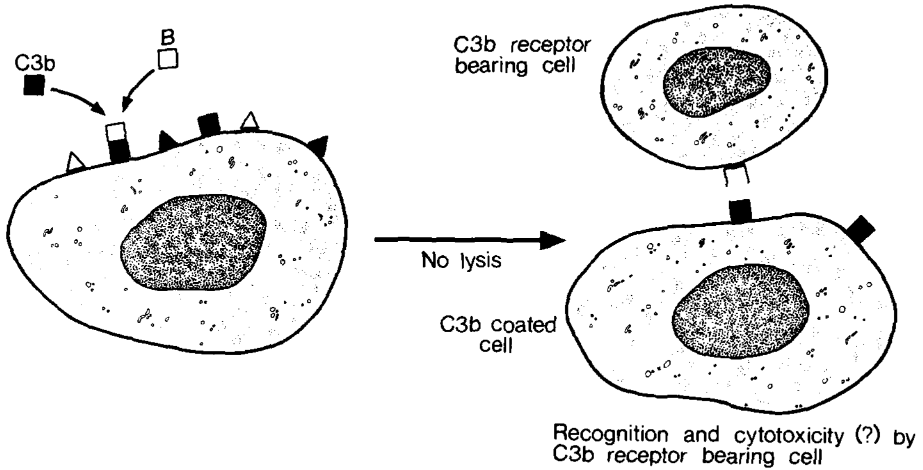
Other enveloped viruses are also associated with activation of the complement pathway although this activation is often dependent on the cells through which the virus has been passaged (Welsh, 1977). Activation of the alternative pathway has been recorded for Newcastle's disease virus (Welsh, 1977) and for Moloney leukaemia virus (Bartholomew et al., 1978) although in the latter case activation was very weak and probably not of biological significance.

5.3 Lysis of cells by antibody and complement

A curious feature of the role of antibody in enveloped virus infections is that its presence is required for lysis of the cells yet it is not responsible for activation of the complement pathway. The presence of the early components of the classical pathway does not appear to be required (Perrin et al., 1976) and lysis of measles infected the HeLa cells can be produced by purified components of the alternative and membrane attack pathways (Sissons et al., 1979). Properidin is also necessary for lysis and whilst neither IgG nor properidin influence the absolute binding of C3 to the target, the rate of binding is increased by IgG and the stability of C3bBb complex is increased by properidin. The combination of these processes may facilitate the formation of the C5 convertase and the subsequent assembly of the membrane attack complex (Sissons et al., 1979).

The paramyxoviruses possess two envelope glycoproteins HN (or H) and F. The HN protein serves as the cell receptor and may possess haemagglutinating and in some cases neuraminidase activity. The F or fusion protein is responsible for syncitium formation. In measles infected cells IgG bound to either the F protein or the H protein can result in lysis (Fig. 4.D). $F(ab')_2$ fragments but not Fab' fragments can also produce lysis. This requirement for divalence indicates that patching of the viral antigen and possibly therefore 'focusing' of the target area is required for effective lysis (Perrin et al., 1976).

C. Alternative pathway of complement, No antibody



D. Alternative pathway of complement plus antibody

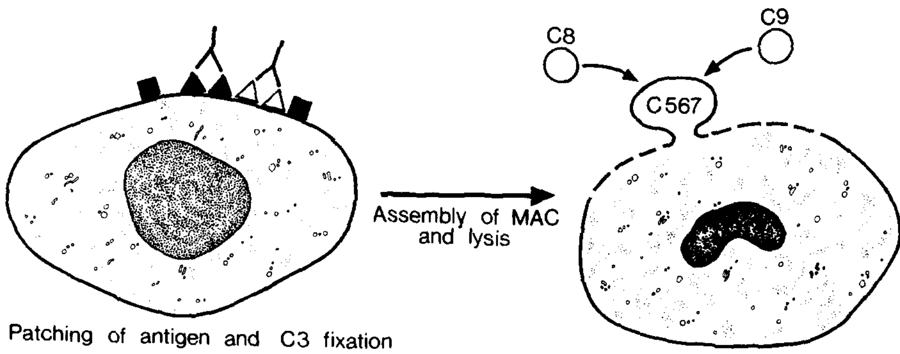


Figure 4.

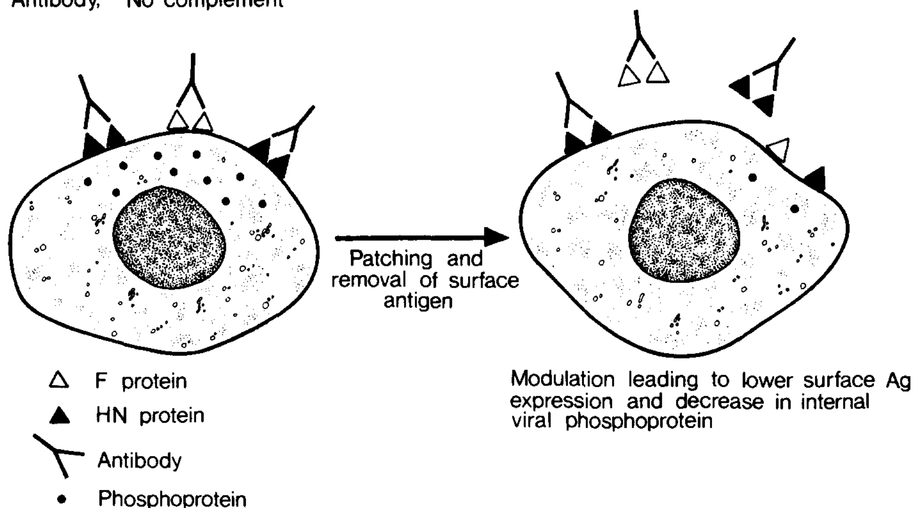
In the absence of a functional or activated complement pathway, the patching of viral antigen continues so that it is eventually stripped off the cell surface (Joseph and Oldstone, 1975). This modulation of the virus antigen on the cell surface results in lower levels of antigen expression and increases the probability that the cell will escape immune destruction. The modulation of surface antigens by antibody in measles infected cells has an additional, intriguing effect on antigen expression. Not only is the expression of surface antigens affected but there is a specific decrease in intracellular viral protein concentrations and a change in protein phosphorylation (Fujinami and Oldstone, 1979). (Fig. 5A). It has not been determined if these changes are due to increased degradation or decreased synthesis but the clear implication is that the immune response can modify the net production of viral proteins. It is possible that these processes may be a more general phenomenon involved in the establishment of latent infections.

The specificity of antibody can also influence the fate of the infected cell. In paramyxovirus infections antibody to the HN (or H) protein can neutralise the virus by preventing virus adsorbtion. Most inactivated vaccines to paramyxoviruses result in the production of anti-HN but not anti-F antibody. In these circumstances the virus is still able to spread from cell to cell through fusion of adjacent plasma membranes since anti-F antibody is necessary to inhibit this activity (Fig. 5B) (Merz et al., 1980). Furthermore in the absence of anti-F antibody continued HN antigen presentation will occur in the form of virus released from the expanding syncitia. Antigen-antibody-complement complexes formed during this process may account for the immunopathological responses that have been observed in respiratory syncitial (RS) and measles virus infections (Chatterji and Mankad, 1977; Kim et al., 1969). Interestingly the severest pneumonia in RS virus infections occurs in children with the highest neutralising antibody titres (Chanock et al., 1970) and RSV induced disease in cattle has been suggested to have an immunopathological component (Kim, 1977).

There is an inherent problem in evaluating the significance of in vitro phenomena in terms of their biological relevance in vivo. Some doubt has been cast on the importance of complement mediated lysis since relatively large amounts of antibody are required to effect lysis. In the case of measles infected cells 6.5×10^7 molecules of IgG per cell were required for 50% lysis (Sissons et al., 1979). Nevertheless good correlation does exist between complement mediated lysis and protection. In feline leukaemia virus infections the presence of complement fixing antibodies to the FeLV transformation antigen FOCMA is strongly associated with

HUMORAL IMMUNE RESPONSE TO PARAMYXOVIRUS INFECTED CELLS

A. Antibody, No complement



B.. Antibody to HN. No Anti-F antibody

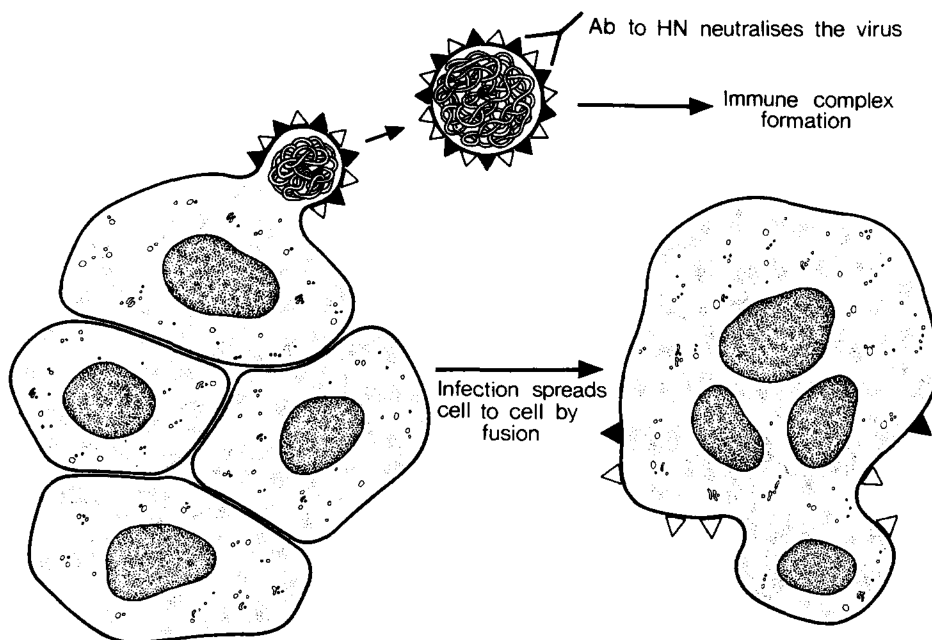


Figure 5.

protection from tumour development (Grant et al., 1980).

5.4 Immunopathology associated with the humoral response

Immunopathology is a feature of many virus diseases but in some, it is the predominant component in their pathogenesis. Most attention has been directed at immune complex disease and important veterinary examples occur in aleutian disease of mink (for review see Porter et al., 1980) equine infectious anaemia (for review see McGuire and Crawford, 1979) and feline infectious peritonitis (Pedersen and Boyle, 1980; Weiss and Scott, 1981).

In these diseases the virus infection persists despite a vigorous humoral response. The continued production of virus antigen in the face of this antibody response results in the formation of immune complexes. Eventually the formation of these complexes overwhelms the ability of the phagocytic system to degrade them, and they become deposited in glomeruli and arteries. Viruses from quite different groups are involved in immune complex disease but there are some interesting parallels in their biology. The antibody produced to the virus may be non-neutralising, as in aleutian disease, and whilst neutralising antibody is produced in EIA infection it is clear that the virus does circulate in non-neutralised virus antibody complexes (McGuire, et al., 1972). In EIA the disease is episodic and each new episode appears to be associated with antigenic variants that are not neutralised by antibody that was effective against viruses present earlier in the course of the disease (Kono, et al., 1973).

A second feature common to these viruses is that they replicate in macrophages, although this is not necessarily the major site of virus production. Entry into these cells may be faciliated by phagocytosis of the virus as an infectious antigen-antibody-complex. Once replication of viruses begins it may reduce the capacity of the macrophage to handle immune complexes which then precipitate and initiate the cycle of tissue injury.

6. ANTIBODY DEPENDENT CELL MEDIATED CYTOTOXICITY

Like complement mediated cytotoxicity, antibody dependent cell mediated cytotoxicity (ADCC) is directed at the virus infected cell. Specificity in the cytotoxic response is provided by IgG bound to the target, whilst the effector component is an Fc bearing mononuclear cell often referred to as a k-cell. The term k-cell is a functional definition and distinct cell types possessing Fc receptors have been reported to have k-cell activity. These

have included monocytes and macrophages (Kohl et al., 1977; Shore et al., 1977) polymorphonuclear leucocytes (Gale and Zighelboim, 1975) and T-cells (Santoli and Koprowski, 1979). In most species the main body of evidence suggests that the most efficient effector of k-cell activity, in the peripheral blood, is an Fc bearing cell in the 'null set' of mononuclear cells, that is, a non-adherent, E-rossette negative, surface Ig^- cell (Greenberg et al., 1973). However, different effector cells may act in different situations and there may be species differences in the effectiveness of cell populations to initiate ADCC. In the dog ADCC activity against distemper infected cells has been produced by a null cell population comparable to the k-cells of man (Ho and Babiuk, 1979) whilst in cattle, ADCC to herpes virus infected cells was mediated most efficiently by neutrophils (Grewal et al., 1977). In the latter case however, it was found that neutrophils could also exert an antiviral effect which was distinct from ADCC and which did not require direct interaction between the neutrophil and the target cell (Rouse et al., 1977).

A feature of ADCC which suggests that it may be relevant in vivo is that relatively little antibody is required to sensitise cells in comparison to complement dependent cytotoxicity. When measles infected HeLa cells are used as targets an estimated 5×10^5 antibody molecules per cell are required for sensitisation, a figure at least one order of magnitude lower than for complement mediated cytotoxicity (Perrin et al., 1977). High concentrations of antibody may even inhibit ADCC, possibly as antigens are modulated from the target cells and the resulting complexes block the Fc receptor of the effector cell (Andersson et al., 1975). The mechanism of lysis produced by the k-cell(s) is not understood but in most systems antibody must first bind to the target cell (Greenberg, 1977). Once this step has occurred the effector cells can interact with the Fc portion of the immunoglobulin molecule and effect the lytic process. In vitro the lytic process develops rapidly, membrane damage being detectable within a few minutes of plating the effector cells (Ziegler and Henney, 1975). During this lytic phase, the kinetics of cell killing (measured by ^{51}Cr release) demonstrate a 'one hit' pattern indicating that only a single effector target cell interation is necessary to produce lysis. In contradistinction to T-cell killing once the effector cell has bound to its target it is inactivated and unable to participte in further cytolytic actions (Ziegler and Henney, 1975).

The signficance of ADCC in vivo is still not clear. The relative sensitivity of the cytotoxic event requiring only a small amount of antibody and the rapidity of the cell killing produced suggest, however,

that ADCC may play a significant role in eliminating virus infected cells in vivo.

7. CELL MEDIATED CYTOTOXICITY

Cell mediated cytotoxicity is directed towards the infected cell and is therefore restricted to enveloped virus infections in which viral antigens are substituted in the cell membrane. Unlike ADCC which requires the presence of both antibody and an effector cell, natural killer cell mediated cytotoxicity (NCMC) and T-cell mediated cytotoxicity only require the presence of activated effector cells.

Cytotoxicity to virus infected cells can be measured in vitro in two principal ways. In microcytoxicity assays adherent target cells are plated in microwells and the ability of lymphocytes to kill these targets over 1 to 2 days is measured by direct counting of remaining viable cells at the end of the assay (Takasugi and Klein, 1970). Whilst this is a sensitive assay it is both time-consuming and subject to considerable variability. Furthermore it does not necessarily distinguish between cytotoxicity and growth inhibition. A more widely used test is the ^{51}Cr release assay (Cerrotini and Brunner, 1974; Doherty et al., 1977). In this system target cells are labelled with ^{51}Cr which is released only slowly from normal cells. When ^{51}Cr labelled, virus infected, cells are incubated with cytotoxic effector cells, membrane lesions are produced in the targets with consequent lysis and release of the label. The extent of release of the label over the 4-6 hours of the assay can be used to quantitate the degree of cytotoxicity. From studies with these in vitro assays it is clear that the lytic process involves direct contact between the effector and target cell, followed (in T-cells) by an activation step associated with the production of membrane damage (Henney, 1973).

7.1 Natural killer cells

Natural killer cells are a population of non-adherent mononuclear cells which exhibit spontaneous cytoxicity against a range of cell lines including normal cells, tumour cells and virus infected cells. The high frequency of these cells in the peripheral blood of man (1-2% of all mononuclear cells) and their state of activation has led to suggestions that they form a first line of defence against neoplastic or virus infected cells (Takasugi et al., 1973; Kiessling et al., 1975).

7.2 The NK cell

An intuitively attractive hypothesis of NK function is that these cells represent the same class of 'Null cell' that is responsible for ADCC and that the NK activity is due to 'pre-arming' of an Fc receptor by antibody. In studies on human and murine NK cells some similarities to K cells have been reported (Kay et al., 1977; Santoni, 1979).

Although NK activity may be found in a non-adherent, mononuclear population in the peripheral blood of man, spleens of mice and the lymphoid compartments of cats (McCarty and Grant, 1980) pigs (Huh et al., 1981) chickens (Lam and Linna, 1977) and other species, it is not certain that these cells are identical to K-cells. Prearming of NK cells with antibody should render natural cell mediated cytoxicity (NCMC) susceptible to blocking by anti-IgG or the $F(ab')_2$ fragment of the anti-immunoglobulin. Whilst anti-immunoglobulin effectively blocks ADCC (Trinchieri et al., 1975) it fails to inhibit or only partially inhibits NCMC (Trinchieri et al., 1978; Kay et al., 1978; Pape et al., 1979). Distinguishing between NCMC and ADCC activity does nevertheless, require the use of careful controls since NK activity is often measured in long assays during which antibody may be secreted in vitro from PBL. For instance the apparent increase in spontaneous cytoxicity of PBL after influenza virus vaccination has been shown to correlate with the ability to produce anti-haemagglutinin antibody in vitro (Greenberg et al., 1979).

A solution to the dichotomy between the similarity of the populations producing NK and ADCC activity and the difference in susceptibility of these responses to anti-immunoglobulin has been suggested by Santoli and Koprowski (1979). They have proposed that ADCC and NCMC are different cytolytic processes mediated by the same population, a suggestion which is supported by the data of Ojo and Wigzell (1978).

Most information on the nature of the human NK cell suggests that it is a non-adherent surface Ig negative cell with an Fc receptor of low avidity. Using human NK cells eluted from their targets, Saksela et al. (1979) found that most rosetted with sheep red blood cells (SRBC) and displayed an acid a-napthyl acetate esterase staining pattern typical of lymphocytes. Santoli and Koprowski (1979) also found a proportion of the NK activity amongst a SRBC rosetting, IgG Fc^+, population suggesting that the human NK cell may belong to the T_g-lymphocyte subset that can display suppressor cell activity (Moretta et al., 1977). Murine NK cells exhibit the Ly5 gene product also found on the surface of thymocytes, but not on B-cells or macrophages, (Cantor et al., 1979). Furthermore they have either no or low

expression of Thy-1 antigen (Heberman et al., 1978) findings which would be consistent with murine NK cells belonging to an early stage in the T-cell lineage (Herberman and Holden, 1978). More recent studies, however, suggest that these Ly5 positive cells may be related to basophils (Galli et al., 1982).

7.3 Genetic control of NK expression

In the mouse significant strain differences in NK activity have been observed and the level of this activity appears to be linked to genes in the major histocompatibility complex (Klein et al., 1978).

Apart from these strain differences a profound selective defect in NK activity has been recorded in homozygous beige mice (Roder and Duwe, 1979). Studies with homozygous beige mice have shown that the rate and number of metastases from a primary tumour are higher in the homozygous bg/bg mouse compared to the heterozygous littermates +/bg which have normal NK activity. Furthermore the extent of metastasis of the tumours correlates with their susceptibility to NCMC.

7.4 Target cell specificity of NK activity

NK cells appear to exhibit cytolytic activity against a wide range of cells but this activity appears to be highest against allogeneic cells (Hannson et al., 1978) and cells cultured in vitro, particularly lymphoid cells (Kiessling et al., 1975). These differences in susceptibility to NCMC of different cell lines does not appear to be related to the acquisition of antigen from the culture media (Haller, 1978).

An important question therefore is the specificity of the recognition event by NK cells. Using a technique in which NK cells were depleted by binding to different target cells Kiessling and Wigzell (1979) found that depletion of NK activity was not specific for a particular target but correlated with susceptibility of the target to NCMC. Similar findings have been recorded for NK activity against virus infected cells; for whilst virus infected cells were more susceptible to NK activity than were their non-infected counterparts, cold competition and target absorption studies suggested that lysis was effected by a population capable of cytotoxicity against a range of target cells. (Santoli et al., 1978; Weston et al., 1981).

7.5 Interferon and NK activity

In addition to their antiviral activity the interferons have a range of immunoenhancing and immunosuppressive effects (Epstein, 1977). Included in the former is the enhancing effect of interferon on NK cell activity. Trinchieri and Santoli (1978) and Trinchieri et al., (1978) observed that short term incubation of lymphocytes in exogenous interferon markedly increased the levels of natural cell mediated cytotoxicity without augmenting ADCC. In similar experiments Herberman et al. (1979) found an augmentation of NK activity by prolonged treatment with exogenous interferon, but in contrast to Trinchieri and Santoli they also noted an enhancement of ADCC.

In addition to responding to exogenous interferon, the NK component of lymphocytes used in cytotoxic assays can be activated by interferon produced endogenously in assays from infected target cells. Consequently caution is required when interpreting the results of ADCC or T-cell mediated cytotoxicity assays against virus infected cells.

A possible regulatory role for interferon in NCMC is suggested by the observation that interferon not only activates NK cells but also induces a protective effect against NCMC in normal uninfected fibroblasts. (Trinchieri and Santoli, 1978).

Although the role of NK cells in virus infections in vivo is open to question, the in vitro data lends support to their role as rapid effector components against enveloped virus infections. Both NCMC and the alternative pathway of complement are activated by virus infected cells without specificity for particular viruses. These processes may represent primordal cellular and humoral arms of the immune system that have been retained as the first line of defence against virus infections.

8. CYTOTOXIC T-CELL RESPONSE

The first indications that cell mediated response is important in recovery from viral infection came from studies of immunodeficient patients. In general patients with hypogammaglobulinaemia can exhibit an apparently normal recovery from infections by enveloped viruses whilst patients with specific T-cell defects often develop serious progressive infections. These clinical observations have been confirmed experimentally. When the thymus is ablated, susceptibility to enveloped viruses increases whilst immune responsiveness to non-enveloped viruses remains near normal (Blanden, 1970; Zisman, 1970; Rager-Zisman and

Allison, 1973).

The induction of cell mediated responses to viral antigens is associated with a number of events that can be monitored including a delayed hypersensitivity response, a proliferation of T-cells and the release of lymphokines including gamma interferon. However, the central event in T-cell mediated immunity is the destrution of virus infected cells by cytotoxic T-cells.

8.1 MHC restriction of cytoxicity

A major development in the study of T-cell mediated immunity was the observation that the cytotoxic activity of murine T-cells was restricted to virus infected target cells bearing the same major histocompatibility (MHC) antigens. The MHC restriction could be demonstrated for both cytotoxic effector functions, which required recognition of the gene products of the K and D region of the MHC, and for T-cell helper activities which were restricted by I region products (Zinkernagel and Doherty, 1974; Doherty and Zinkernagel, 1975; Zinkernagel and Doherty, 1979). A number of important theoretical and practical consequences have stemmed from these observations

At the theoretical level the appreciation of how T-cells "see" antigen has been influenced by the discovery of MHC restriction and a number of models for the recognition of antigen by T-cells have been proposed. Each of these models must take account of several factors. Firstly, MHC restriction is not simply due to an interaction between identical MHC molecules on the effector and target cell. Indeed identity between the MHC products of the effector and target cell is not necessary under certain circumstances. The T-cell 'learns' to recognise as "self" the histocompatibility antigens displayed on the thymic epithelium during T-cell development in that organ. Consequently when irradiated A strain mice are reconstituted with (A x B) F1 bone marrow cells, the cytotoxic T-cells which develop in the chimaeras only recognise and lyse virus infected cells of strain A. Thus, although the peripheral lymphoid tissue contains both F1 T-cells and F1 antigen presenting cells, capable of presenting antigen in association with both A and B MHC products, response is restricted, primarily to the MHC A product present on the irradiation resistant thymic epithelium. (Zinkernagel et al., 1978a).

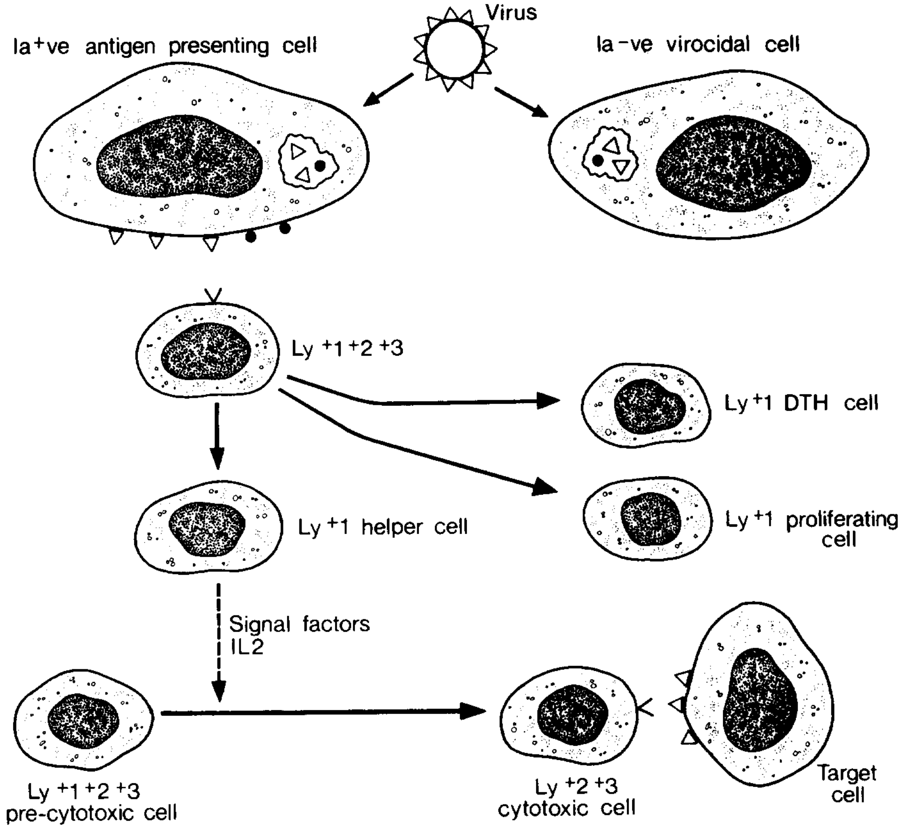
Secondly, as pioneers of transplant surgery soon discovered, MHC products are strong antigens. It has been estimated that up to 10% of

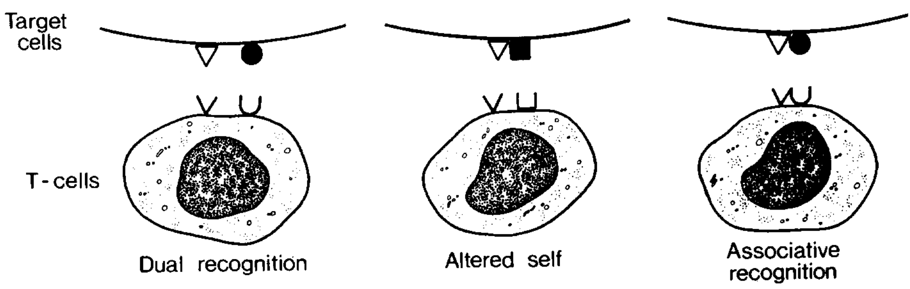
T-cells may specifically react with any foreign haplotype (Dutton, 1966; Swain et al., 1979). This high degree of MHC polymorphism and associated alloreactivity presumably has an important role in governing the response to antigens. Thirdly, it is becoming clear that T-cells specific for a particular antigen bear idiotypes identical to antibodies specific for the same antigen (Binz and Wigzell, 1977). At least part of the T-cell antigen receptor repetoire is, therefore, probably encoded by VH genes. However, unlike the VH genes expressed on B-cells which, it is believed, can be the products of somatic mutation during ontogeny, the VH genes of T-cells may be limited to those encoded in the germ line. This may account for the failure to observe an increase in the affinity of the T-cell receptor for antigen during the development of the immune response (Krawinkel et al., 1978).

Within the constraints given above a number of models of antigen recognition by T-cells are possible. The extreme examples are the 'altered self' and the 'dual recognition' models (Fig. 6). Early discussions of the T-cell receptor centred on the altered self model in which it was assumed that antigen associated with the MHC product creating a conformational change in the latter. This altered MHC product could then be recognised by the single T-cell receptor.

In the dual recognition model it is envisioned that separate receptors exist for the MHC product and the antigen. An intermediate form of this model, the associative recognition model, involves a single receptor recognising an unmodified MHC product together with the antigen (Fig. 6). Until recently dual receptor models have predominated (Cohn and Epstein, 1978; Zinkernagel et al., 1978b; Williamson, 1980) but this issue is still far from resolved and there has been a recent resurgence of the altered self hypothesis (Matzinger, 1981). Whichever model is correct it is apparent that the recognition of the antigen in association with the MHC governs the extent of the immune response. The polymorphism of the MHC products in a species may have been maintained by the selective pressure of a variety of infectious diseases on the population, each evoking a distinct level of response with a given MHC product.

MHC-restriction of T-cell cytotoxicity has been observed in other species including man. In species of veterinary interest indications of MHC restriction have been observed in the chicken in immunity to Rous-sarcoma virus infected cells (Wainberg, et al., 1974). The apparent absence of MHC restriction in the cytotoxic reaction of cattle to IBR and vaccinia virus infections (Rouse and Babiuk, 1977) and the similar findings by Ho et al. (1978) in dogs are of interest but should be viewed with





△ Viral antigen
● Normal MHS product
■ Altered MHS product

Figure 6.

caution. Firm conclusions on the absence of restriction are not possible unless both defined effector cell populations and MHC typed cells are available. Progress in this area, in the domestic animals, should be facilitated by the ability to infect lymphocytes in vitro with a number of viruses and by the ability to grow effector T-cell populations in long term culture. (Onions et al., 1980; Rojko et al., 1980). In addition progress is now being made in defining the MHC products of a number of domestic animals including the dog (Vriesendorp, 1979).

8.2 The development of the cytotoxic response

The development and regulation of the cytotoxic response requires interactions between distinct T-cell subsets and involves both T-cell help and suppression. The induction of the immune response is also dependent on macrophages (Zisman et al., 1970) which function as antigen presenting cells. This latter function is carried out by an Ia^+ group of cells which may be distinct from Ia^- macrophages involved in removing antibody-complement-virus complexes. (Finberg and Benacerraff, 1981). The T-lymphocyte subsets involved in the murine immune response can be distinguished on the basis of their Lyt antigen profile. Lyt $1^+2^-3^-$ T-cells see antigen in association with I region MHC products and provide T-cell help, whilst those with an Lyt $1^-2^+3^+$ phenotype are cytotoxic effector cells which recognise antigen in the context of K and D region MHC products.

The early stages of the induction of the immune response involves the Lyt $1^+2^-3^-$ cell interacting with virus antigen presented on the surface of an Ia^+ macrophage. The helper cell requires two signals for its activation. One is a specific signal provided by recognition of the antigen with the I region product, whilst the second is probably a non-specific lymphokine, interleukin 1 (IL1) released by the antigen presenting cell. Once activated, the helper cell can produce interleukin 2 (IL2). This factor may have a role in enhancing the differentiation of Lyt $1^-2^+3^+$ precursor (Hardt et al., 1980) but its principal function is acting as the second signal in the induction of the cytotoxic response. In an analagous fashion to the generation of T-cell help the cytotoxic T-cell must recognise the viral antigen and an MHC product, in this case it recognises K and D MHC structures. Once the cytotoxic lymphocyte has interacted with antigen it expresses a receptor for IL2 and becomes sensitive to IL2 released from the helper cell. Interleukin 2 appears to be necessary for activation of the cytotoxic T-cell (Wagner et al., 1980)

and as its former name of "T-cell growth factor" suggests it is important in expanding this cytotoxic pool.

8.3 Antigens recognised by cytotoxic T-cells

The cytotoxic T-cell response appears early after infection and this activity coupled with gamma interferon production by T-cells are the important determinants in recovery from primary infection by enveloped viruses. Neutralising antibody, although delayed in its onset in comparison to the cytotoxic response, persists for longer and consequently forms the main barrier to reinfection. This may pose different selection pressures on the determinants recognised by cytotoxic T-cells in comparison to those recognised by antibody. Selection for variant serologically defined antigens on viruses is likely to occur since only variant antigens will successfully challenge a protective humoral response, whilst in the primary infection handled by the T-cell system, variant antigens are of less significance (Zinkernagel and Rosenthal, 1981). In the light of these observations it is not surprising to find that cytotoxic T-cells often show cross reactivity between serologically defined virus subgroups. In some instances this may be because type specific reactions in the cytotoxic assay are obscured by group specific cytotoxic events.

An apparent example of this phenomenon is the cross reactivity of murine cytotoxic T-cells for target cells infected with different strains of influenza viruses. Originally it was thought that this was due to recognition by the T-cells of membrane-bound, group-specific, matrix protein. However quantitation of the amount of matrix protein on cell membranes makes this an unlikely explanation (Hackettt et al., 1980) indicating that the T-cells may express a broad specificity for the antigenically variable haemagglutinin molecule of influenza virus.

9. CONCLUSIONS

One of the most significant achievements of medicine in the last century has been the control of major virus diseases by vaccination. Since the work of Jenner and Pasteur progress has often been achieved through inspired empericism with little reference to "theoretical immunology". Nevertheless, there has always remained a core of important diseases that have not been amenable to vaccination. Control of these diseases will undoubtedly require new insights into the protective immune response. Furthermore the prospect of a new generation of vaccines based on synthetic

peptides and recombinant DNA technology will pose new problems for the immunologist.

10. REFERENCES

Anderson, T., Stejskal, V and Härfast, B, (1975). An in vitro method for study of human lymphocyte cytotoxicity against mumps-virus-infected target cells. J. Immunol., 114: 237-243.

Ashe, W.K., Mage, M. and Notkins, A.L. (1969). Kinetics of neutralisation of sensitized Herpes Simplex Virus with Antibody Fragments. Virology, 37: 290-293.

Bartholomew, R.M., Esser, A.F. and Müller-Eberhard, H.J. (1978). Lysis of oncornaviruses by human serum. J. exp. med., 147: 844-853

Berry, D.M. and Almeida J.D. (1968). The morphological and biological effects of various antisera on avian infectious bronchitis virus. J. Gen. Virol., 3: 97-102.

Binz, H. and Wigzell, H. (1977). Antigen binding idiotypic T lymphocyte receptors. Contemp. Top Immunobiol., 7: 113.

Blanden, R.V. (1970). Mechanisms of recovery from a generalized viral infection : mousepox. J. Expt. Med., 132: 1035-1054.

Blanden, R.V., Hapel, A.J. and Jackson, D.C. (1976). Mode of action of Ir genes and the nature of T cell receptors for antigen. Immunochemistry, 13: 179-191.

Bubbers, J.E. and Lilly, F. (1977) Selective incorporation of H2-antigenic determinants into Friend virus particles.. Nature, 266: 458-459.

Budzko, D.B., Lachmann, P.J. and McConnell, I. (1976). Activation of the alternative complement pathway by lymphoblastoid cell lines derived from patients with Burkitt's Lymphoma and Infectious Mononucleosis. Cell Immunol., 22: 98-109.

Burns, W.H. (1975). Viral Antigens. In: Viral Immunology and Immunopathology. Ed. A.L. Notkins. pp.43-56. Academic Press, N.Y.

Cantor, H., Kasai, M., Shen, F.W., Leclerc, J.C. and Glimcher, L. (1979). Immunogenic Analysis of "Natural killer" Activity in the mouse. Immun. Rev., 44: 3-12.

Cerrotini, J.C. and Brunner, K.T. (1974). Cell mediated cytotoxicity, allograft rejection and tumour immunity. Adv. Immunol., 18: 16-132.

Chanock, R.M., Kapikain, A.Z., Mills, J., Kim, H.W. and Parrott, R.H. (1970). Influence of immunological factors in respiratory syncitial virus disease. Arch. Environ. Health, 21: 347-355.

Chatterji, M. and Mankad, V. (1977). Failure of attenuated viral vaccine in prevention of atypical measles. J. Am. Med. Ass., 238: 2635.

Cohn, M. and Epstein, R. (1978). T-Cell inhibition of humoral responsiveness II. Theory on the role of restrictive recognition in immune regulation. Cellular Immunology, 39: 125-153.

Cooper, N.R., Jensen, F.C., Welsh, Jr., R.M. and Oldstone, M.B.A. (1976). Lysis of RNA tumour viruses by human serum : Direct antibody-indpendent triggering of the classical complement pathway. J. exp. Med., 144: 970-984.

Daniels, C.A., Boros, T., Rapp, H.J., Synderman, R. and Notkins, A.L. (1970). Neutralization of sensitized virus by purified components of complement. Proc. Natl. Acad. Sci., 65: 528-535.

Dimmock, N.J. and Possee, R.D. (1981). How does neutralised virus become non-infectious? J. Supramolecular Structure Supplement, 5: 378.

Doherty, P.C. and Zinkernagel, R.M. (1975). H-2 compatibility is required for T-cell mediated lysis of target cells infected with lymphocyte choriomeningitis virus. J. exp. med., 141: 502-507.

Doherty, P.C., Palmer, J. and Zinkernagel, R.M. (1977). Experimental analysis of effector thymus derived lymphocytes in viral infections. In: "Methods in Virology" (K. Maramorsch and H. Koprowski eds.). Vol. 6, pp. 29-57. Academic Press, New York.

Dutton, R.W. (1966). Spleen cell proliferation in response to homologous antigens studied in congenic resistant strains of mice. J. exp. med. 123: 665-671.

Epstein, L.B. (1977). The effects of interferons on the immune response in vitro and in vivo. In: Interferons and their actions ed. Stewart W. II CRC Press Cleveland.

Ertl, H. and Ada, G.L. (1981). Roles of influenza virus infectivity and glycosalation of viral antigen for recognition of target cells by cytolytic T lymphocytes. Immunobiol., 158: 239-253.

Essex, M., Klein, G. and Snyder, P. et al. (1971). Correlation between humoral antibody and regression of tumours induced by feline sarcoma virus. Nature., 233: 195-196.

Finberg, R. and Benacerraff, B. (1981). Induction, control and consequences of virus specific cytotoxic T cells. Immunological Rev., 58: 157-180.

Fujinami, R. and Oldstone, M.B.A. (1979). Antiviral antibody reacting on the plasma membrane alters measles virus expression inside the cell. Nature, 279: 529-530.

Gale, R.P. and Zighelboim, (1975). Polymorphonuclear leucocytes in antibody-dependent cellular cytotoxicity. J. Immunol., 114: 1047-1051.

Galli, S.J., Dvorak, A.M., Ishizaka, T., et al. (1982). A cloned cell with NK function resembles basophils by ultrastructure and expresses IgE receptors. Nature, 298: 288-290.

Grant, C.K., Harris, D., Essex, M., Pickard, D., Hardy, W., de Noronha, F. (1980). Protection of cats against feline leukaemia virus-positive and virus-negative tumours by complement-dependent antibody. J. Natl. Cancer Inst., 64: 1527-1534.

Greenberg, A.H., Haidson, L., Shen, L. and Roitt, I.M. (1973). Antibody dependent cell mediated cytotoxicity due to a nall lymphoid cell. Nature (New Biol.), 242: 111-113.

Greenberg, S.B., Criswell, B.S., Six, H.R. and Couch, R.B. (1977). Lymphocyte cytotoxicity to influenza virus-infected cells. J. Immunol., 119: 2100-2106.

Greenberg, S.B., Six, H.R., Drake, S. and Couch, R.B. (1979). Cell cytoxicity due to specific influenza antibody production in vitro after recent influenza antigen stimulation. Proc. Nat. Acad. Sci. 76: 4622-4626.

Grewal, A.S., Rouse, B.T. and Babiuk, L. (1977). Mechanisms of resistance to herpes viruses : Comparison of the effectiveness of different cell types in mediating antibody-dependent cell mediated cytotoxicity. Infection and Immunity, 15: 698-703.

Griffin, J.A. and Compans, R.W. (1979). Effect of cytochalisin B on the maturation of enveloped viruses. J. Exp. Med., 150: 379-391.

Hackett, C.J., Askonas, B.A., Webster, R.G. and van Wyke, K. (1980). Quantitation of influenza virus antigens on infected target cells and their recognition by cross reactive cytotoxic T cells. J. exp. med., 151: 1014-1025.

Haller, O. (1978). Mouse natural cell-mediated cytotoxicity to human leukaemia : Independence of serum source used for target-cell culturing and cytotoxic assay. J. Natl. Cancer Inst., 60: 1433-1438.

Hansson, M., Kärrek, K., Bakacs, T., Kiessling, R. and Klein, G. (1978). Intra- and interspecies reactivity of human and mouse natural killer (NK) cells). J. Immunol., 121: 6-12.

Hardt, C., Pfizenmair, K., Rollinghoff, M., Klein, J. and Wagner, H. (1980). Alloreactive and 112 restricted Lyt 23 cytotoxic T lymphocytes derive from a common pool of antecendant Lyt 123 precursor. J. exp. Med., 152, 1413-1418.

Henney, C. (1973). On the mechanism of T-cell mediated cytolysis. Transplant Rev., 17: 37-70.

Herberman, R.B. and Holden, H.T. (1978). Natural cell mediated immunity. Adv. Cancer Res., 27: 305-377.

Herberman, R.B., Nunn, M.E. and Holden, H.J. (1978). Low density of Thy 1 antigen on mouse effector cells mediating natural cytotoxicity against tumour cells. J. Immunol., 121: 304-309.

Herberman, R.B., Djeu, J.Y., Kay, H.D., et al. (1979). Natural killer cells : Characteristics and regulation of activity. Immun. Rev. 44: 43-70.

Hierholzer, J.C. and Dowdle, W.R. (1970). Immunological basis of adenovirus 8-9 cross reaction. Journal of Virology, 6: 782-787.

Ho, C.K., Babiuk, L.A. and Rouse, B.T. (1978). Immune effector cell activity in canines : Failure to demonstrate genetic restriction in direct antiviral cytotoxicity. Infec. and Immun., 19: 18-26.

Ho, C.K. and Babiuk, L.A. (1979). Immune mechanisms against canine distemper I Identification of K cell against canine distemper virus infected target cells in vitro. Immunology, 37: 231-239.

Huh, N.D., Kim, Y.B. and Amos, D.B. (1981). Natural killing (NK) and antibody-dependent cellular cytotoxicity (ADCC) in specific pathogen free miniature swine and germ free piglets. Two distinct effector cells for NK and ADCC. J. Immunol., 127: 2190-2193.

Joseph, B.S. and Oldstone, M.B.A. (1975). Immunologic injury in measles virus infection II Suppression of immune injury through antigenic modulation. J. exp. med., 142: 864-876.

Kay, H.D., Bonnard, G.D., West, W.H. and Herberman, R.B. (1977). A functional comparison of human Fc receptor-bearing lymphocytes active in natural cytotoxicity and antibody dependent cellular cytotoxicity. J. Immunol., 118: 2058-2066.

Kay, H.D., Bonnard, G.D. and Herberman, R.B. (1978). Evaluation of the role of IgG antibodies in human natural cell mediated cytotoxicity against the myeloid cell line K562. J. Immunol., 122: 675-685.

Kiessling, R., Klein, E. and Wigzell, H. (1975). Natural killer cells in the mouse I Cytotoxic cells with specificity for mouse Moleny leukemic cells. Specificity and distribution according to genotype. Eur. J. Immunol., 5: 112-117.

Kiessling, R. and Wigzell, H. (1979). An analysis of the murine NK cell. Immun. Rev. 44: 165-208.

Kim, J.C.S. (1977). Immunological injury in "shipping fever" pneumonia of cattle. Vet. Rec., 100: 109-111.

Kim, H.W., Canchola, J.G., Brandt, C.D., Pyles, G., Chanock, R.M., Jensen, K. and Parrott, R.H. (1969). Respiratory syncitial virus disease in infants despite prior administration of antigenic inactivated vaccine. Am. J. Epid., 89: 422-434.

Kjellen, L. and Pereira, H.G. (1968). Role of adenovirus antigens in the induction of virus neutralizing antibody. J. Gen. Virol. 2: 177-185.

Klein, G.O., Klein, G., Kiessling, R. and Karrek, (1978). H-2 Associated control of natural cytoxicity and hybrid resistance against RBL-5. Immunogenetics, 6: 561-569.

Kohl, S., Starr, S.E., Oleske, J.M., Shore, S.L., Ashman, R.B. and Nahmias, A.J. (1977). Human monocyte-macrophage-mediated antibody dependent cytotoxicity to herpes simplex virus infected cells. J. Immunol., 118: 729-735.

Kono, Y., Fukunaga, Y. and Kobayashi, K. (1973). Nat. Inst. Anim. Health Q., 13: 173-181.

Krawinkel, V., Cramer, M., Melchers, I., Imanishi-kari, S and Rajewsky, K. (1978). Isolated hapten-binding receptor of sensitized lymphocytes. III Evidence for idiotypic restriction of T-cell receptors. J. exp. med., 147: 1341-1347.

Lachmann, P.J. and Nicol, P.A.E. (1973). Reaction mechanism of the alternative pathway of complement fixation. Lancet, 1973 (1), 465-467.

Lachman, P.J. and Hobart, M.J. (1979). The genetics of the complement system. In : Human genetics possibilities and realities. Ciba Symposium 66 (NS) Excerpta Medica.

Lafferty, K.J. (1963). The interaction between virus and antibody. Virology, 21: 76-90.

Lam, K.M. and Linna, T.J. (1977). Protection of newly hatched chickens from Marek's disease (JMV) by normal spleen cells from older animals. In: Advances in comparative leukaemia research ed. Bentvelzen et al./Elsevier North Holland.

Lundstedt, C. (1969). Interaction between antigenically different cells. Virus induced cytotoxicity by immune lymphoid cells in vitro. Acta. Pathol. Microbiol. Scand., 75: 139-152.

McCarty, J.M. and Grant, C.K. (1980). Cytotoxic effector cell populations in cats. In: Feline Leukaemia Virus. Eds. W.D. Hardy, Jr., M. Essex and A.J. McClelland. pp. 203-210. Elsevier North Holland.

McConnell, I., Klein, G. and Lunt, T.F. (1978). Activation of the alternative complement pathway by human B cell lymphoma lines is associated with Espstein-Barr virus transformation of the cells. Eur. J. Immunol. 8: 453-458.

McGuire, T.C., Crawford, T.B. and Henson, J.B. (1972). Equine infectious anaemia : detection of infectious virus-antibody complexes in the serum. Immunol. Commun., 1: 545-551

McGuire, T.C. and Crawford, T.B. (1979). Immunology of a persistent retrovirus infection equine infectious anaemia. Adv. in Vet. Sci. and Comp. Med., 23: 137-159.

Mandel, B. (1967). The interaction of neutralized poliovirus with HeLa cells II. Elution penetration and uncoating. Virology, 31: 248-259.

Mandel, B. (1976). Neutralisation of poliovirus: a hypothesis to explain the mechanism and the one-hit character of the neutralisation. Virology, 69: 500-510.

Matzinger, P. (1981). A one receptor view of T-cell behaviour. Nature, 292: 497-501.

Merz, D.C., Scheid, A. and Choppin, P.W. (1980). Importance of antibodies to the fusion glycoprotein of paramyxoviruses in the prevention of spread of infection. J. exp. med., 151: 275-288.

Meynell, G.G. and Meynell, E.W. (1970). In: Theory and practice in experimental bacteriology. 2nd Edition. London: Cambridge University Press.

Moretta, L., Webb, S.R., Grossi, C.E., Lydyard, P.M. and Cooper, M.D. (1977). Functional analysis of two human T-cell subpopulations : help and suppression of B-cell responses by T-cells bering receptors for IgM or IgG. J. exp. med., 146: 184-200.

Müller-Eberhard, H.J. and Götze, O. (1972). C3 proactivator convertase and its mode of action. J. Exp. Med., 135: 1003-1008.

Neil, J.C., Smart, J.E., Hayman, M. and Jarrett, O. (1980). Polypeptirdes of feline leukemia virus: A glycosalated gag-related protein is released into culture fluids. Virology, 105: 250-253.

Norrby, E. (1971). Adenoviruses. In: comprative virology pp. 105-134. Eds. K. Maramorosch and E. Kurstak. N.Y. Academic Press.

Norrby, E. and Wadell, G. (1972). The relationship between the soluble antigens and the virion of adenovirus type 3. Virology, 48: 757-765.

Ojo, E. and Wigzell, H. (1978). Natural killer cells may be the only cells in normal mouse lymphoid cell populations endowed with cytolytic ability for antibody-coated tumour target cells. Scand. J. Immunol., 7: 297-306.

Oldstone, M.B.A., Cooper, N.R. and Larson, D.L. (1974). Formation and biologic role of polyoma virus antibody complexes. J. exp. med., 140: 549-565.

Oldstone, M.B.A. (1975). Virus neutralisation and virus-induced immune complex disease. Progress in Medical Virology, 19: 84-119.

Onions, D.E., Testa, N. and Jarrett, O. (1980) Growth of FeLV in haemopoietic cells in vitro. In: Feline Leukaemia Virus pp. 513-516. Eds. W.D. Hardy, Jr., M. Essex and A.J. McClelland. Elsevier North Holland. N.Y.

Oroszlan, S. and Gilden, R.V. (1970). Immune virolysis: Effect of antibody and complement on C-type RNA virus. Science, 168: 1478-1480.

Pangburn, M.K., Morrison, D.C., Schreiber, R.D. and Müller-Eberhard, H.J. (1980). Activation of the alternative complement pathway: recognition of surface structures on activators by bound C3b. J. Immunol., 124: 977-982.

Pape, G.R., Troye, M., Aexlsson, B. and Perlmann, P. (1979). Simultaneous occurrence of immunoglobulin-dependent and immunoglobulin-independent mekchanisms in natural cytotoxicity of human lymphocytes. J. Immunol., 122: 2251-2260.

Pedersen, N.C. and Boyle, J.F. (1980). Immunologic phenomena in the effusive form of feline infectious peritonitis. Am. J. Vet. Res., 41: 868-876.

Perrin, L.H., Joseph, B.S., Cooper, N.R. and Oldstone, M.B.A. (1976). Mechanism of injury of virus infected cells by anti-viral antibody and complement : Participation of IgG, $(Fab')_2$ and the alternative complement pathway. J. exp. med., 143: 1027-1041.

Perrin, L.H., Tishon, A. and Oldstone, M.B.A. (1977). Immunologic injury in measles virus infection. J. Immunol., 118: 282-290.

Petranyi, G., Kiessling, R., Povey, S., Klein, G., Herzenberg, L. and Wigsell, H. (1976). The genetic control of natural killer activity and its association with in vivo resistance against a moloney lymphoma isograft. Immunogenetics., 3: 15-28.

Pettersson, U. (1971). Structural proteins of adenoviruses. VI. On the antigenic determinants of the hexon. Virology, 43: 123-136.

Porter, D.D., Austin, A.E. and Porter, H.G. (1980). Aleutian disease of mink. Advances in Immunology, 29: 261-286.

Portelle, D., Bruck, C., Mammerickx and Burny, A. (1981). Natural antibodies to BLV gp51 are reactive against the carbohydrate moiety of the glycoprotein. In: Modern Trends in Human Leukaemia IV. Eds. R. Gallo, et al. Springer-Verlag. N.Y.

Powell, P.C., Payne, L.N., Frazier, J.A. and Rennie, M. (1974). T Lymphoblastoid cell lines from Marek's disease lymphomas. Nature, 251: 79-80.

Radwan, A.I. and Crawford, T.B. (1974). The mechanisms of neutralization of sensitized equine arteritis virus by complement components. J. Gen. Virol., 25: 229-237.

Rager-Zisman, B. and Allison, A.C. (1973). Effects of immunosuppression on Coxsackie B-3 virus infection in mice, and passive protection by circulating antibody. J. Gen. Virol., 19: 339-351.

Roder, J.C. and Duwe, A. (1979). The biege mutation in the mouse selectively impairs natural killer cell function. Nature, 278: 451-453.

Roder, J.C., Rosen, A., Fenyo, E.M. and Troy, F.A. (1979). Target-effector interaction in the natural killer cell system : Isolation of target structures. Proc. Natl. Acad. Sci., 76: 1405-1409.

Rojko, J.L., Hoover, E.A., Finn, R.L. and Olsen, R.G. (1980). Interactions between the feline leukaemia virus (FeLV) and isolated feline lymphocytes. In Feline Leukaemia Virus, pp. 187-194 Ed. W.D. Hardy, Jr., M. Essex and A.J. McClelland. Elsevier North Holland, N.Y.

Rojko, J.L., Hoover, E.A., Quackenbush, S.L. and Olsen, R.G. (1981). Latency and reactivation of feline leukemia virus infection. In: Advances in Comparative Leukaemia Research 1981. Ed. D.S. Yohn and J.R. Blakeslee. pp. 225-226. Elsevier Biomedical.

Rouse, B.T. and Babiuk, L.A. (1977). The direct antiviral effect cytoxicity by bovine lymphocytes is not restricted by genetic incompatibility of lymphocytes and target cells. J. Immunol, 118: 618-624.

Rouse, B.T., Wardley, R.C., Babiuk, L.A. and Mukkur, T.K.S. (1977). The role of neutrophils in antiviral defense - in vitro studies on the mechanism of antiviral inhibition. J. Immunol., 118: 1957-1961.

Saksela, E., Timonen, T., Ranki, A. and Häyry, P. (1979). Morphological and functional characterisation of isolated effector cells responsible for human natural killer activity to fetal fibroblasts and to cultured cell line targets. Immun. Rev., 44: 71-123.

Santoli, D. and Koprowski, H. (1979). Mechanisms of activation of human natural killer cells. Immunological Rev., 44: 125-163.

Santoli, D., Trinchieri, G. and Lief, F.S. (1978). Cell mediated cytotoxicity against virus-infected target cells in humans. J. Immunol., 121: 526-531.

Santoni, A., Herberman, R.B. and Holden, H.T. (1979). Correlation between natural and antibody-dependent cell mediated cytotoxicity against tumor targets in the mouse. J. Natl. Cancer Inst., 63: 995-1003.

Shore, S.L., Melewicz, F.M. and Gordon, D.S. (1977). The mononuclear cell in human blood which mediates antibody dependent cellular cytotoxicity to virus infected target cells. J. Immunol., 118: 558-566.

Sissons, J.G.P., Schreiber, R.D., Perrin, L.H., Oldstone, M.B.A., Cooper, N.R. and Müller-Eberhard (1979). Lysis of measles virus-infected cells by the purified cytolytic alternative complement pathway and antibody. J. exp. med., 150: 445-454.

Sissons, J.G.P., Oldstone, M.B.A. and Schreiber, R.D. (1980). Antibody independent activation of the alternative complement pathway by measles virus-infected cells. Proc. Natl. Acad. Sci., 77: 559-562.

Swain, S.L., Panfili, P.R., Dutton, R.W. and Lekvovits, I. (1979). Frequency of allogeneic helper T-cells responding to whole H-2 differences to an H-2k difference alone. J. Immunol., 123: 1062-1067.

Takasugi, M. and Klein, E. (1970). A microassay for cell mediated immunity. Transplantation, 9: 219-227.

Takasugi, M., Mickey, M.R. and Terasaki, P.I. (1973). Reactivity of lymphocytes from normal persons on cultured tumor cells. Cancer Res., 33: 2898-2904.

Takasugi, M., Akira, D., Takasagi, J. and Mickey, M.R. (1977). Specificities in human cell mediated cytotoxicity. J. Natl. Cancer Inst., 59: 69-75.

Trinchieri, G., Baumann, P., De Morchi, M. and Tokes, Z. (1975). Antibody dependent cell mediated cytotoxicity in humans. I. Characterization of the effector cell. J. Immunol., 115: 249-255.

Trinchieri, G., Santoli, D. and Koprowski, H. (1978). Spontaneous cell-mediated cytotoxicity in humans. Role of interferon and immunoglobulins. J. Immunol., 120: 1849-1855.

Trinchieri, G. and Santoli, D. (1978). Antiviral activity induced by culturing lymphocytes with tumor-derived or virus transformed cells. J. exp. med., 147: 1314-1333.

Vriesendorp, H.M. (1979). Applications of transplant immunology in the dog. Adv. in veterinary Sci. and comp. med., 23: 229-265.

Wagner, H., Hardt, C., Heegk., Pfizenmaier, K., Solbach, W., Bartlett, R., Stockinger, H. and Rollinghoff, M. (1980). T-T-cell interactions during cytotoxic T lymphocyte responses. Immunol. Rev. 51: 215:255.

Wainberg, M.A., Markson, Y., Weiss, D.N. and Doljanski, F. (1974). Cellular immunity against Rous sarcomas of chickens. Preferential reactivity against autochthonous target cells as determined by lymphocyte adherence and cytotoxicity tests in vitro. Proc. Natl. Acad. Sci. USA, 71: 3565-3569.

Warner, N.L., Woodruff, M. and Burton, R.G. (1977). Inhibition of the growth of lymphoid tumours in syngeneic athymic (nude) mice. Int. J. Cancer, 20: 146-155.

Weiss, R.C. and Scott, F.N. (1981). Pathogenesis of feline infetious peritonitis : Pathological changes and immunofluorescence. Am. J. Vet. Res., 42: 2036-2048.

Welsh, R.M., Cooper, N.R., Jensen, F.C. and Oldstone, M.B.A. (1975). Human serum lyses RNA tumour viruses. Nature, 257: 612-614.

Welsh, R.M. (1977). Host cell modification of lymphocytic choriomeningitis virus and Newcastle disease virus altering viral inactivation by human complement. J. Immunol., 118: 348-354.

Weston, P.A., Jensen, P.J., Levy, N.L. and Koren, H.S. (1981). Spontaneous cytotoxicity against virus-infected cells. Relationship to NK against uninfected cell lines and to ADCC. J. Immunol., 126: 1220-1224.

Whaley, K. and Ruddy, S. (1976). Modulation of the alternative complement pathway by β1H globulin. J. exp. med., 144: 1147-1163.

Williamson, A.R. (1980). Three-receptor, clonal expansion model for selection of self recognition in the thymus. Nature, 283: 527-532.

Witter, R.L., Stephens, E.A., Sharma, J.M. and Nazerian, K. (1975). Demonstration of a tumor-associated surface antigen in Marek's disease. J. Immunol., 115: 177-183.

Ziegler, H.K. and Henney, C. (1975). Antibody-dependent cytolytically active human leukocytes : An analysis of inactivation following in vitro interaction with antibody coated target cells. J. Immunol., 115: 1500-1504.

Zinkernagel, R.M. and Doherty, P.C. (1974). Immunological surveillance against altered self components by sentitized T-lymphocytes in lymphocytic choriomeningitis. Nature, 251: 547-548.

Zinkernagel, R.M., Callahan, G.N., Klein, J. and Dennert, G. (1978a). Cytotoxic T-cells lean specificity for self H-2 during differentiation in the thymus. Nature, 271: 251-253.

Zinkernagel, R.M., Callahan, G.N., Althage, A., Cooper, S. Klein, P.A. and Klein, J. (1978b). On the thymus in the differentiation of "H-2 self-recognition" by T-cells : Evidence for dual recognition. J. exp. med., 147: 882-896.

Zinkernagel, R.M. and Doherty, P.C. (1979). MHC-Restricted cytotoxic T cells. Advances in Immunology, 27: 51-177.

Zinkernagel, R.M. and Rosenthal, K.L. (1981). Experiments and speculation on antiviral specificity of T and B cells. Immunological Rev. 58: 131-155.

Zisman, B., Hirsch, M.J. and Allison, A.C. (1970). Selective effect of anti-macrophage serum, silica and anti-lymphocyte serum in pathogensis of herpes virus infection of young adult mice. J. Immunol., 104: 1155-1159.