ANTIGEN-SPECIFIC THYMUS CELL FACTORS IN THE GENETIC CONTROL OF THE IMMUNE RESPONSE TO POLY-(TYROSYL, GLUTAMYL)-POLY-D, L-ALANYL--POLY-LYSYL*

BY M. J. TAUSSIG, EDNA MOZES, AND RONIT ISAC

(*From the Immunology Division, Department of Pathology, University of Cambridge, Cambridge, England, and the Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel*)

In recent years the use of antigens of restricted structural heterogeneity has led to the discovery and study of specific immune response (Ir)[1] genes (1–5). Immune responsiveness to many immunogens has been found to be linked to major histocompatibility loci (1–7). Classical examples of histocompatibility-linked immune response genes are the poly-L-lysine (PLL) gene in guinea pigs (2–4) and the $Ir-1$ gene in mice (1–3, 5–8). The PLL gene controls responsiveness to poly-L-lysine, poly-L-arginine, copolymers composed of L-glutamic acid and L-lysine, and to hapten conjugates of these polypeptides, and the $Ir-1$ gene regulates determinant-specific responses to multichain synthetic polypeptides. Genetically determined high, medium, and low levels of response to these antigens have been shown to be characteristic of various inbred strains of these animals. Clearly a key question is the cellular nature of the expression of this genetic control. The immunogens to which the response is controlled by $Ir-1$ are thymus-dependent, requiring the cooperation of thymus-derived (T) and bone marrow-derived (B) cells for efficient antibody response. In principle, therefore, a gene locus such as $Ir-1$ could exert a controlling influence on T cells or B cells or both. Although a certain dogma is frequently expressed to the effect that $Ir-1$ control is invariably T-cell associated, the matter is often a subject of some controversy (1). The example we have studied is one such controversial case. The antigen is the multichain synthetic polypeptide poly-L-(Tyr, Glu)-poly-D, L-Ala--poly-L-Lys, abbreviated (T, G)-A--L, which in mice stimulates a high response in strains of histocompatibility $(H-2)$ type b, and a low response in strains of $H-2$ type k (inter alia) (6–8). Some evidence has been interpreted as support for the existence of a T-cell defect, with normal B-cell capability, in low responder mice. This includes the production of high anti-(T, G)-A--L responses in low responder strains by coupling the antigen to a

---

[1] *Abbreviations used in this paper:* Ir, immune response; PFC, plaque-forming cell; (Phe,G)-A--L, poly-L(Phe, Glu)-poly-D,L-Ala--poly-L-Lys; (T,G)-A--L, poly-L(Tyr, Glu)-poly-D,L-Ala--poly-L-Lys; (T,G)-Pro--L, poly-L(Tyr, Glu)-poly-L-Pro--poly-L-Lys.

protein carrier (9); and the similarity between the response pattern in low responders and thymectomized high responders (10, 11). Other lines of investigation, on the other hand, suggest the opposite conclusion, namely that it is the B cell and not the T cell which is the functionally defective partner. This is inferred from experiments in which the numbers of T- and B-cell precursors available for (T, G)-A--L have been titrated by "limiting dilution analysis" (5, 12, 13), and is supported by others in which cells of high and low responder origin have been mixed and the response pattern of the allogeneic mixtures determined (13).

In order to resolve this problem, two pieces of experimental data are crucial, namely (a) a direct comparison of the ability of high and low responder T cells to respond to (T, G)-A--L, and (b) a comparison of the ability of high and low responder B cells to respond to (T, G)-A--L in the presence of equivalent T-cell stimulation. It is important in obtaining this information to avoid possible "allogeneic effects" (14, 15) which might occur if cells of different H-2 origins are mixed, and to use congenic strains of mice so that the genetic difference between strains can be strictly limited to the histocompatibility loci to which Ir-1 is intimately linked (1). We have approached the problem by a new method, namely by the use of active T-cell products termed T-cell "factors" (16). A system has recently been developed in which it is possible to obtain from T cells certain antigen-specific factors which are able to replace the requirement for T cells in thymus-dependent immune responses in vivo (16). It has already been shown using Balb/c mice that T cells educated to (T, G)-A--L produce such factors in vitro, and that these factors can completely replace an otherwise absolute requirement for T cells in the primary response to (T, G)-A--L of B cells transferred into irradiated, syngeneic recipients (16). Furthermore, the factors have been shown to be true T-cell products, by inhibiting their production with anti-θ serum, and to be specific for the inducing antigen, in this case (T, G)-A--L. A crucial property of the factors is that allogeneic combinations of factors and B cells are as effective in cooperation as syngeneic combinations, and that nonspecific allogeneic effects are avoided.

The properties of T-cell factors have been applied to the critical problems in genetic control posed above, using mice of the congenic strains C3H.SW (H-2^b) and C3H/HeJ (H-2^k), high and low responders respectively to (T, G)-A--L. The results of the present study show that (T, G)-A--L-educated T cells of both C3H.SW and C3H/HeJ origins are equally capable of producing active cooperative factors in response to (T, G)-A--L. However, only high responder B cells could be stimulated by (T, G)-A--L and T-cell factors in vivo. The data indicate that the genetically controlled difference between the strains is expressed solely in the B-cell population, and not in the T cells.

Materials and Methods

Antigens.—The following multichain synthetic polypeptides were used: (a) poly-L(Tyr, Glu)-poly-D,L-Ala--poly-L-Lys, abbreviated as (T,G)-A--L, batch no. 1383; (b) poly-L-

(Phe, Glu)-poly-D, L-Ala--poly-L-Lys, abbreviated as (Phe, G)-A--L, batch no. 929; (c) poly-L(Tyr, Glu)-poly-L-Pro--poly-L-Lys, abbreviated as (T, G)-Pro--L, batch no. 935. The synthesis and characterization of such immunogens have been described previously (17–19).

Animals.—Mice of the congenic strains C3H.SW and C3H/HeJ, bred at the Experimental Animal Unit, The Weizmann Institute of Science, Rehovot, were used. C3H.SW are H-2^b and are high responders to (T, G)-A--L; C3H/HeJ are low responders, H-2^k (7).

Preparation and Test of T-Cell Factors.—The preparation of T-cell factors from educated T cells in vitro and their test with B cells in vivo have been described in detail elsewhere (16). In brief the methods used were as follows. Educated or primed T cells were prepared in both strains by transferring 10^8 thymocytes into each of a group of irradiated (800 R of ^{60}Co gamma irradiation) syngeneic recipients, and immunizing each animal intraperitoneally 1 day later with 10 μg (T, G)-A--L in Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.). 7 days following immunization, the spleens of these mice, containing the educated T cells, were removed and cell suspensions were prepared in minimal Eagle's medium. The suspensions contained two–three spleen equivalents per ml (approximately 10^7 viable lymphocytes per ml). (T, G)-A--L to a concentration of 1 μg/ml was then added and the suspensions cultured in small petri dishes, under an atmosphere of 5% CO_2 at 37°C for 6–8 h. At the end of this time, the cells were removed by centrifugation and the supernate, containing T-cell factors, was mixed with bone marrow cells and (T, G)-A--L and transferred into irradiated (800 R) recipients, syngeneic for the bone marrow donors. Each recipient received one spleen equivalent of the supernatant T-cell factor, 10^7 bone marrow cells, and 10 μg (T, G)-A--L in solution. 12 days following transfer, the recipients were killed and their spleens removed and assayed for direct plaque-forming cells (below). In each experiment, appropriate controls, such as transfer of B cells and (T, G)-A--L alone, or of B cells, (T, G)-A--L and 10^8 normal thymocytes, were included, as indicated in the results. In general, 15 recipients were present in each group.

Hemolytic Plaque-Forming Cells Assay.—Direct plaque forming cells (PFC) in the spleens of recipient mice were determined using antigen-coated sheep red blood cells (SRBC). (T, G)-Pro--L-coated SRBC were used when the immunogen was (T, G)-A--L, since the antibody response to (T, G)-A--L is almost wholly directed to the (Tyr, Glu) determinants. In experiments using (Phe, G)-A--L, SRBC coated with the homologous antigen were used for the PFC assay. The polypeptides were attached to SRBC by incubating equal volumes of packed SRBC, chromium chloride [10 mg/ml for coating with (T, G)-Pro--L and 2 mg/ml for coating with (Phe, G)-A--L)] and the antigen (10 mg/ml) for 5 min at room temperature. After coating, the SRBC were washed three times in phosphate-buffered saline (PBS) and diluted to a final concentration of 10% in PBS. The assay was performed as previously described (16), according to Jerne et al. (20).

RESULTS

Thymus-Dependence of Primary Response to (T, G)-A--L *on Transfer, and Magnitude of Differences Between Strains.*—The results given in Table I show that the primary direct PFC response to (T, G)-A--L given by cells transferred into irradiated recipients is highly thymus-dependent, and that an approximately 20-fold difference in size of response exists between C3H.SW high responders and C3H/HeJ low responders. 10^7 bone marrow cells of either strain were transferred into lethally irradiated syngeneic recipients together with 10 μg (T, G)-A--L in solution, and either with or without 10^8 normal syngeneic thymocytes. The 12-day direct PFC responses in the spleens of the recipients are shown in Table I. It is evident that, in the absence of T cells, C3H.SW produce no more than a small background response to (T, G)-A--L,

TABLE I

Thymus-Dependence of the Primary Response to (T, G)-A--L on Transfer, and Magnitude of the Interstrain Difference in Response

Cells transferred into irradiated recipients	Mean PFC per spleen*	
	Log₁₀	(Anti-log)
B cells (C3H.SW)‡	2.5391	(346)
T cells (C3H.SW)§	—	(0)
B cells (C3H.SW) + T cells (C3H.SW)	3.9351	(8,612)
Standard error	0.1360	
B cells (C3H/HeJ)‡	2.0253	(106)
T cells (C3H/HeJ)§	—	(0)
B cells (C3H/HeJ) + T cells (C3H/HeJ)	2.6128	(412)
Standard error	0.2100	

* Geometric means.
‡ 10^7 Bone marrow cells.
§ 10^8 normal thymocytes.

Direct anti-(T, G)-A--L PFC responses in irradiated (800 R) C3H.SW and C3H/HeJ mice 12 days after receiving transfer of syngeneic B cells, T cells, or a mixture of the two, together with 10 μg (T, G)-A--L in solution. Results as log₁₀ geometric means (anti-log values shown); standard errors for each group shown. 15 mice per group.

but this is increased 20–30 times by the addition of T cells. The primary IgM response is therefore highly thymus-dependent under these conditions. In C3H/HeJ, however, the background B-cell response to (T,G)-A--L is barely significantly increased by the presence of T cells. Clearly, a very large difference in optimal IgM response to (T, G)-A--L thus exists between the strains.

Production of Cooperative T-Cell Factors by C3H.SW and C3H/HeJ-Educated T Cells.—Thymocytes of both C3H.SW and C3H/HeJ strains were educated to (T, G)-A--L and then cultured, separately, in vitro with (T, G)-A--L for 6–8 h, as already described in the methods. The cells were then removed by centrifugation and supernatant factors produced by both were mixed (separately) with bone marrow cells of C3H.SW, high responder, origin, and (T, G)-A--L and transferred into lethally irradiated C3H.SW recipients. Controls were included in which T-cell produced factors were replaced by normal T cells. Table II shows the 12-day PFC responses to (T, G)-A--L in the spleens of the recipients. It is clear that both C3H.SW and C3H/HeJ (T, G)-A--L-educated T cells produce a soluble factor in vitro which can almost totally replace the cooperative activity of 10^8 normal thymocytes in vivo.

Various conditions for the production of T-cell factors, already described in a previous report (16), were confirmed, namely that specifically educated T cells are required and that the educating antigen must be present in the culture. Moreover, the factors are antigen-specific in their action. Table III, for example, shows that cooperating factors prepared to (T, G)-A--L did not affect the response of B cells to a non-cross-reacting antigen such as sheep red cells.

TABLE II

Ability of T-cell Factors Produced by High and Low Responder T Cells to Cooperate with High Responder B Cells

Cells and factors transferred into irradiated recipients	Mean PFC per spleen*	
	Log₁₀	(Anti-log)
B cells (C3H.SW)‡	2.4564	(286)
B cells (C3H.SW) + T cells (C3H.SW)§	4.0174	(10,412)
B cells (C3H.SW) + factor (C3H.SW)‖	3.9159	(8,240)
B cells (C3H.SW) + factor (C3H/HeJ)‖	3.8921	(7,800)
Standard error	0.1610	

* Geometric means.
‡ 10^7 bone marrow cells.
§ 10^8 normal thymocytes.
‖ 1 spleen equivalent.

Direct anti-(T, G)-A--L PFC responses in irradiated (800 R) C3H.SW mice 12 days after receiving transfer of syngeneic B cells, 10 μg (T, G)-A--L, and either syngeneic T cells or T-cell factors (C3H.SW or C3H/HeJ) produced in vitro by (T, G)-A--L educated T cells. Results as log₁₀ geometric means (anti-log values shown); standard error shown. 15 mice per group.

TABLE III

Specificity of a T-Cell Factor Produced to (T, G)-A--L

Cells and factors transferred into irradiated recipients	Mean PFC per spleen*	
	Log₁₀	(Anti-log)
B cells (C3H.SW) + SRBC	2.3579	(228)
B cells (C3H.SW) + T cells (C3H.SW) + SRBC	4.3143	(20,618)
B cells (C3H.SW) + factor (C3H.SW)‡ + SRBC	2.4829	(304)
Standard error	0.1421	

* Geometric means.
‡ T-cell factor identical with that in Table II.

Direct anti-SRBC PFC responses in irradiated (800 R) C3H.SW mice 10 days after receiving transfer of syngeneic B cells, 0.1 ml 10% SRBC, and either syngeneic T cells or syngeneic T-cell factor produced in vitro by (T, G)-A--L-educated T cells and identical with that in Table II. Results as log₁₀ geometric means (anti-log values shown); standard error shown. 15 mice per group.

A crucial property of the T-cell factors, utilized in this experiment, is that it is possible to mix factors and B cells of different genetic origin, without risk of nonspecific stimulation or inhibition due to allogeneic effects (M. J. Taussig, manuscript in preparation; see also following sections). Moreover, allogeneic combinations—such as H-2^k factor with H-2^b cells, and vice versa—are as effective in cooperation as syngeneic combinations. This is not always true of allogeneic T cell-B cell combinations (21) and represents an important advantage of using T-cell factors (see also Table V). The most significant point to

emerge from these results was that no interstrain differences either in the ability of educated T cells to produce cooperating factors, or in the activity or efficiency of the factors produced, could be detected.

Ability of C3H.SW and C3H/HeJ B cells to Respond to Cooperative T-Cell Factors and (T, G)-A--L.—Having demonstrated that both C3H.SW and C3H/HeJ educated T cells release active cooperating factors on culture in vitro, it was possible to compare the abilities of B cells of the two strains to respond to (T, G)-A--L in the presence of the T-cell factors. Table IV shows the results of such an experiment. Factors were prepared from educated T cells of

TABLE IV

Abilities of B Cells of High and Low Responder Strains to Cooperate with T Cell Factors

Cells and factors transferred into irradiated recipients	Mean PFC per spleen*	
	Log₁₀	(Anti-log)
B cells (C3H.SW)‡	1.5798	(38)
B cells (C3H.SW) + factor (C3H.SW)§	3.8357	(6,850)
B cells (C3H.SW) + factor (C3H/HeJ)§	3.6125	(5,788)
Standard error	0.1024	
B cells (C3H/HeJ)‡	1.3617	(23)
B cells (C3H/HeJ) + factor (C3H.SW)§	1.9685	(93)
B cells (C3H/HeJ) + factor (C3H/HeJ)§	2.0374	(109)
Standard error	0.1266	

* Geometric means.

‡ 10^7 bone marrow cells.

§ 1 spleen equivalent.

Direct anti-(T, G)-A--L PFC responses in irradiated (800 R) C3H.SW and C3H/HeJ mice 12 days after receiving transfer of syngeneic B cells, T-cell factors (C3H.SW or C3H/HeJ) produced in vitro by (T, G)-A--L-educated T cells, and 10 μg (T, G)-A--L in solution. Results as log₁₀ geometric means (anti-log values shown); standard errors shown. 15 mice per group.

each strain as above, and transferred, separately, with B cells of either C3H.SW or C3H/HeJ origin, and (T, G)-A--L, into irradiated recipients syngeneic with the bone marrow donors. It is seen that excellent cooperation was achieved, as before, between C3H.SW (high responder) B cells and both C3H.SW and C3H/HeJ factors. By contrast, no such cooperation was achieved when C3H/HeJ (low responder) B cells were used with the identical factors. Thus a clear distinction between high and low responder B cells can be made on the criterion of responsiveness to identical T-cell factors. (It may also be pointed out that this result tends to rule out the possibility that T-cell factors can provoke allogeneic effects, i.e., nonspecific stimulation, when combined with allogeneic B cells). The basis of low responsiveness to (T, G)-A--L in C3H/HeJ can therefore be characterized as an inability to respond to stimulation by T cells and antigen.

Responsiveness of C3H/HeJ and C3H.SW B Cells to (Phe, G)-A--L and T-Cell Factors.—It was an essential control for the system to show that C3H/HeJ B cells could be made to respond to another thymus-dependent antigen to which that strain is a high responder, when transferred together with the appropriate T-cell factors. C3H/HeJ are high responders to a closely related synthetic polypeptide, (Phe, G)-A--L. Accordingly, T-cell factors to (Phe, G)-A--L were prepared in both C3H/HeJ and C3H.SW strains (also a high responder to this antigen) and transferred together with C3H/HeJ or C3H.SW B cells and (Phe, G)-A--L into irradiated recipients as described above. Table V shows that excellent cooperative IgM responses were obtained to (Phe, G)-A--L with C3H/HeJ as well as C3H.SW B cells in this case. Thus the nonresponsiveness of C3H/HeJ B cells to stimulation by T-cell factors is antigen-specific, and is limited, as far as can be judged, to the antigen to which the strain is genetically a low responder, (T, G)-A--L. Table V also illustrates the ease with which T-cell factors may be mixed with B cells of different strain origin and give successful cooperation: C3H.SW T-cell factors cooperated as efficiently with C3H/HeJ B cells as with C3H.SW B cells in the transfer response to (Phe, G)-A--L. As already noted, allogeneic cells do not always cooperate effectively (21), and this represents a significant advantage in using specific T-cell factors.

TABLE V

Cooperation between B Cells and T-Cell Factors in the Response to (Phe, G)-A--L

Cells and factors transferred into irradiated recipients	Mean PFC per spleen*	
	Log$_{10}$	(Anti-log)
B cells (C3H/HeJ)‡	2.1004	(126)
B cells (C3H/HeJ) + T cells (C3H/HeJ)§	3.8334	(6,814)
B cells (C3H/HeJ) + factor (C3H/HeJ)‖	3.7168	(5,210)
B cells (C3H/HeJ) + factor (C3H.SW)‖	3.7993	(6,300)
Standard error	0.1633	
B cells (C3H.SW)	2.4150	(260)
B cells (C3H.SW) + T cells (C3H.SW)	3.8646	(7,321)
B cells (C3H.SW) + factor (C3H.SW)	3.8143	(6,522)
B cells (C3H.SW) + factor (C3H/HeJ)	3.7767	(5,980)
Standard error	0.1520	

* Geometric means of direct anti-(Phe, G)-A--L PFC.

‡ 10^7 bone marrow cells.

§ 10^8 normal thymocytes.

‖ 1 spleen equivalent.

Direct anti-(Phe, G)-A--L PFC responses in irradiated (800 R) C3H/HeJ and C3H.SW mice 12 days after receiving transfer of syngeneic B cells, with either T cells or T-cell factors (C3H/HeJ or C3H.SW) produced in vitro by (Phe, G)-A--L-educated T cells, and 10 μg (Phe, G)-A--L in solution. 15 mice per group.

DISCUSSION

The results described here show that (T, G)-A--L-educated T cells of both the C3H.SW and C3H/HeJ strains can be stimulated by antigen in vitro to produce cooperating factors which can replace the requirement for T cells in a primary response to (T, G)-A--L in vivo. Similar factors have already been described in Balb/c mice (16). While the nature and properties of these factors are the subjects of continuing investigation, it is known that they are non-dialyzable and are sensitive to destruction by proteolytic enzymes (M. J. Taussig, manuscript in preparation). That the factors are indeed produced by T cells has been proven by the use of anti-θ serum to inhibit their production in vitro (16). Moreover, they cannot be removed on anti-light chain immuno-adsorbents, and would therefore appear not to be conventional immunoglobulins (M. J. Taussig, manuscript in preparation). Their most important functional characteristic is that they are specific for the inducing antigen, a property which distinguishes them from various nonspecific T-cell products released as a result of allogeneic stimulation or other means (14, 15, 22, 23). In the light of these properties and the efficiency with which the factors cooperate with B cells in the presence of antigen, it seems reasonable to assume that the release of specific T-cell factors and their collaboration with B cells is a normal means whereby T cell-B cell cooperation is effected in vivo. The ability of the T cells of different strains to produce cooperating factors in response to antigen in vitro is therefore likely to be a meaningful measure of their capacity to recognize and respond to antigen under more physiological conditions in vivo. These assumptions are the rationale for the experiments described here. Educated T cells of high and low responder strains were compared for their ability to produce factors capable of cooperating with B cells in the response to (T, G)-A--L; no interstrain differences were found. On the other hand, when B cells of the two strains were compared for their antibody responses to (T, G)-A--L in the presence of active specific T-cell factors of either strain, only high responder B cells responded satisfactorily, while low responder B cells gave consistently negative results. Therefore the conclusion must be drawn that the genetically controlled difference between C3H.SW and C3H/HeJ mice in respect of their responsiveness to (T, G)-A--L lies in the ability of their respective B cells to recognize or respond to antigen in the presence of T-cell factors. In short, the genetic defect is expressed at the level of the B cells, not the T cells.

This conclusion is in basic agreement with results obtained by two other techniques. Limiting dilution analysis has been used to compare the numbers of T and B-cell precursors in high and low responders to (T, G)-A--L (5, 12, 13). High and low responder thymocytes were indistinguishable by this technique, whereas high responder bone marrow cell precursor frequencies were significantly higher than those for the low responder. Confirmatory results have also been obtained using allogeneic mixtures of T and B cells from the congenic

strains C3H.SW and C3H/HeJ (13). Whereas allogeneic cell transfers can be criticized on the grounds of likely interference by allogeneic effects, it has been repeatedly demonstrated and is confirmed here that T-cell factors of one strain and B cells of another can be mixed together with impunity. There is no evidence of nonspecific activation or inhibition as a result of the allogeneic combination of factors and cells of different strains, and B cells are always transferred into syngeneic irradiated recipients. Furthermore there is no difficulty in cooperation across an *H-2* barrier as there is for T-cell and B-cell combinations (21); *H-2^k* factors and *H-2^b* cells cooperated as effectively as syngeneic combinations. Another possibility that has been ruled out is nonspecific stimulation by "bystander" cells, i.e., a small percentage of T cells which may be present in bone marrow cell preparations (15). If the T-cell factors turn out to be associated with *H-2* antigens, they could conceivably be recognized by allogeneic bystander T cells and thus trigger an allogeneic effect (and it is indeed difficult to see how allogeneic stimulation could occur by any other means in this case). However, recent experiments showing the strict antigen-specificity of cooperation by T-cell factors even in allogeneic combination with B cells, have excluded this possibility (M. J. Taussig, manuscript in preparation).

While some evidence has been gathered to build a circumstantial case that the primary genetic defect in low responders to (T, G)-A--L is expressed in T cells (9–11, 24, 25), it can be adequately explained by an alternative hypothesis, namely that the primary genetic defect in low responders is an inability of B cells to recognize and respond to an antigen-specific T-cell signal. For the purposes of this model, B cells of high and low responders are considered identical in all other respects, including precursor number and recognition of antigen by surface immunoglobulin receptor. T cells of both strains are assumed to be identical in the characteristics of their response. On recognizing antigen, T cells respond by releasing a soluble, antigen-specific factor with which B cells must interact, in addition to their own recognition of antigen, for thymus-dependent antibody responses to be triggered. It is this specific interaction of cooperative T-cell factor and B cell which, on the basis of the direct evidence reported here, is believed to be at fault in the low responders. In terms of the Bretscher-Cohn model for cooperation (26), the B cells are unable to respond to the "second signal" provided by a specific T-cell-produced "associative antibody."

The observations made in this report measure T-cell and B-cell responsiveness directly and show that the function controlled by the *Ir-1* locus is associated with the B-cell response to antigen. It is extremely encouraging that an independent technique has very recently indicated that low responder (parental) B cells are unable to cooperate with high responder (F$_1$)T cells in an *H-2* linked response under conditions where allogeneic effects, etc., could also be excluded (27). It is becoming clear, however, that different antigens will reveal different possible means whereby the immune response is affected by genetic influences. Indeed there are cases where there is little doubt that the T cell

rather than, or as well as, the B cell is the cell type affected by genetic control. Good examples of these are the PLL gene in guinea pigs (1–4), the response to derivatives of multichain polyalanine in SJL mice (13, 28, 29), and the response of SWR mice to multichain polyproline and its conjugates (30). In all these cases, there is a clear inability to recognize the carrier aspect of the immunogen. It has therefore been suggested that wherever such a lack of carrier recognition is involved, a T-cell defect will be implicated, but that where a purely determinant-specific low response occurs , the genetic deficiency will be reflected in the B cells (13, 30). We are presently examining the ability of SJL educated T cells to produce cooperative factors to (T, G)-A--L and are able to confirm, from our preliminary data, that they are unable to produce any significant cooperative activity. This is in clear contrast to the results described here for the determinant-specific defect in C3H/HeJ, and strongly supports the hypothesis that it will be possible to correlate T-cell and B-cell defects with carrier and determinant-specific recognition defects respectively. These observations can easily be accommodated in the model described here for interaction between T-cell factors and immunocompetent cells, since T-cell stimulation itself may well also require recognition of a specific T-cell product (T-T cooperation); such recognition would then also be subject to genetic control as it is in B cells as suggested here.

SUMMARY

The genetic control of the antibody response to a synthetic polypeptide antigen designated poly-L(Tyr, Glu)-poly-D, L-Ala--poly-L-Lys [(T, G)-A--L] has been studied in congenic high responder C3H.SW $(H-2^b)$ and low responder C3H/HeJ $(H-2^k)$ strains of mice. This response is controlled by the $Ir-1$ gene and is $H-2$ linked. The method employed was to study the ability of specifically primed or "educated" T cells of each strain to produce cooperative factors for (T, G)-A--L in vitro. Such factors have been shown to be capable of replacing the requirement for T cells in the thymus-dependent antibody response to (T, G)-A--L in vivo. The T-cell factors produced were tested for their ability to cooperate with B cells of either high or low responder origin by transfer together with bone marrow cells and (T, G)-A--L into heavily irradiated, syngeneic (for bone marrow donor) recipients. Direct anti-(T, G)-A--L plaque-forming cells were measured later in the spleens of the recipients. The results showed that (a) educated T cells of both high and low responder origin produced active cooperative factors to (T, G)-A--L, and no differences between the strains in respect to production of T-cell factors could be demonstrated; and (b) such factors, whether of high or low responder origin, cooperated efficiently with B cells of high responder origin only, and hardly at all with B cells of low responder origin. The conclusion was drawn that the cellular difference between the two strains lies in the responsiveness of their B cells to specific signals or stimuli received from T cells. As far as could be discerned by the methods

used, no T-cell defect existed in low responder mice and the expression of the controlling *Ir-1* gene was solely at the level of the B cells in this case.

REFERENCES

1. McDevitt, H. O., and M. Landy, editors. 1972. Genetic Control of Immune Responsiveness. Academic Press, Inc., New York.

2. Benacerraf, B., and H. O. McDevitt. 1972. Histocompatibility-linked immune response genes. *Science (Wash. D. C.).* **175**:273.

3. McDevitt, H. O., and B. Benacerraf. 1969. Genetic control of specific immune responses. *Adv. Immunol.* **11**:31.

4. Benacerraf, B., H. G. Bluestein, I. Green, and L. Ellman. 1971. Specific immune response genes of guinea pigs. *Prog. Immunol.* **1**:487.

5. Mozes, E., and G. M. Shearer. 1972. Genetic control of immune responses. *Curr. Top. Microbiol. Immunol.* **59**:167.

6. McDevitt, H. O., and M. L. Tyan. 1968. Genetic control of the antibody response in inbred mice. Transfer of response by spleen cells and linkage to the major histocompatibility (*H-2*) locus. *J. Exp. Med.* **128**:1

7. McDevitt, H. O., and A. Chinitz. 1969. Genetic control of the antibody response: relationship between immune response and histocompatibility (H-2) type. *Science (Wash. D. C.).* **163**:1207.

8. McDevitt, H. O., and M. Sela. 1965. Genetic control of the antibody response. I. Demonstration of determinant-specific differences in response to synthetic polypeptide antigens in two inbred strains of mice. *J. Exp. Med.* **122**:517.

9. McDevitt, H. O. 1968. Genetic control of the antibody response. III. Qualitative and quantitative characterisation of the antibody response to (T, G)-A--L in CBA and C57 mice. *J. Immunol.* **100**:485.

10. Grumet, F. C. 1972. Genetic control of the immune response. A selective defect in immunologic (IgG) memory in nonresponder mice. *J. Exp. Med.* **135**:110.

11. Mitchell, G. F., F. C. Grumet, and H. O. McDevitt. 1972. Genetic control of the immune response. The effect of thymectomy on the primary and secondary responses of mice to poly-L-(Tyr, Glu)-poly-D, L-Ala--ply-L-Lys. *J. Exp. Med.* **135**:126.

12. Shearer, G. M. 1972. H. O. McDevitt and M. Landy, editors. Genetic Control of Immune Responsiveness. Academic Press, Inc. New York and London. 38.

13. Lichtenberg, L., E. Mozes, G. M. Shearer, and M. Sela. 1974. The role of thymus cells in the immune response to poly(Tyr, Glu)-polyDLAla--polyLys as a function of the genetic constitution of the mouse strain. *Eur. J. Immunol.* In press.

14. Katz, D. H., and B. Benacerraf. 1972. The regulatory influence of activated T cells on B cell responses to antigen. *Adv. Immunol.* **15**:1.

15. Katz, D. H. 1972. The allogeneic effect on immune responses: model for regulatory influences of T lymphocytes on the immune system. *Transplant. Rev.* **12**:141.

16. Taussig, M. J. 1974. T cell factor which can replace the activity of T cells in vivo. *Nature (Lond.).* **248**:234.

17. Sela, M., S. Fuchs, and R. Arnon. 1962. Studies on the chemical basis of the anti-

genicity of proteins. V. Synthesis, characterisation and immunogenicity of some multichain and linear polypeptides containing tyrosine. *Biochem. J.* **85**:223.

18. Fuchs, S., and M. Sela. 1964. Antigenicity of some new synthetic polypeptides and polypeptidyl gelatins. *Biochem. J.* **93**:566.

19. Jaton, J.-C., and M. Sela. 1968. Role of optical configuration in the immunogenicity and specificity of synthetic antigens derived from multichain polyproline. *J. Biol. Chem.* **243**:5616.

20. Jerne, N. K., and A. A. Nordin. 1963. Plaque formation in agar by single antibody-producing cells. *Science (Wash. D. C.).* **140**:405.

21. Katz, D. H., T. Hamaoka, and B. Benacerraf. 1973. Cell interactions between histoincompatible T andB lymphocytes. II. Failure of physiologic cooperative-interactions between T and B lymphocytes from allogeneic donor strains in humoral response to hapten-protein conjugates. *J. Exp. Med.* **137**:1405.

22. Waldmann, H., and A. J. Munro. 1972. T cell dependent mediator in the immune response. *Nature (Lond.).* **243**:356.

23. Schimpl, A., and E. Wecker. 1972. Replacement of T cell function by a T cell product. *Nat. New Biol.* **237**:15.

24. Ordal, J. C., and F. C. Grumet. 1972. Genetic control of the immune response. The effect of graft-vs.-host reaction on the antibody response to poly-L-(Tyr, Glu)-poly-D,L-Ala--poly-L-Lys in nonresponder mice. *J. Exp. Med.* **136**:1195.

25. Hämmerling, G. J., T. Masuda, and H. O. McDevitt. 1973. Genetic control of the immune response. Frequency and characteristics of antigen binding cells in high and low responder mice. *J. Exp. Med.* **137**:1180.

26. Bretscher, P., and M. Cohn. 1970. A theory of self-nonself discrimination. *Science (Wash. D. C.).* **169**:1042.

27. Katz, D. H., T. Hamaoka, M. E. Dorf, P. H. Maurer, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. IV. Involvement of the immune response (*Ir*) gene in the control of lymphocyte interactions in responses controlled by the gene. *J. Exp. Med.* **138**:734.

28. Shearer, G. M., E. Mozes, and M. Sela. 1972. Contribution of different cell types to the genetic control of immune response as a function of the chemical nature of the polymeric side chain (poly-L-prolyl and poly-DL-alanyl) of synthetic immunogens. *J. Exp. Med.* **135**:1009.

29. Mozes, E., G. M. Shearer, E. Maron, R. Arnon, and M. Sela. 1973. Cellular studies of the genetic control of immune response towards the loop region of lysozyme. *J. Immunol.* **111**:1429.

30. Mozes, E., and M. Sela. 1974. The role of the thymus in a genetically controlled defect of the immune response at the carrier level. *Proc. Natl. Acad. Sci. U.S.A.* In press.