GENETIC CONTROL OF THE IMMUNE RESPONSE

THE EFFECT OF THYMECTOMY ON THE PRIMARY AND SECONDARY ANTIBODY RESPONSE OF MICE TO POLY-L(TYR,GLU)-POLY-D,L-ALA--POLY-L-LYS*

BY GRAHAM F. MITCHELL,‡ F. CARL GRUMET,§ AND HUGH O. MCDEVITT‖

(From the Division of Immunology, Department of Medicine, Stanford University School of Medicine, Stanford, California 94305)

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The *H-2*–linked *immune response-1* (*Ir-1*)[1] gene controls murine antibody responses to the synthetic polypeptide poly-L(Tyr, Glu)-poly-D,L-Ala--poly-L-Lys [(T,G)-A--L] (1). It was demonstrated in the preceding paper, using immunization with antigen in aqueous solution, that both high responder (*H-2*^{b}) and low responder (*H-2*^{k}) mice produce equal levels of IgM anti-(T,G)-A--L antibody after a primary antigen challenge (2). With secondary and tertiary challenges, however, only the high responder strain develops immunologic memory, producing high titers of IgG antibody. These studies suggested that the *Ir-1* gene effect is exerted during the induction of IgG antibody formation. Furthermore, in earlier studies utilizing a regimen of immunization with antigen in complete Freund's adjuvant (CFA) followed by a secondary challenge with aqueous antigen, it was shown that: (*a*) low responder (nonresponder) mice will produce high titers of anti-(T,G)-A--L antibody if the immunization is carried out with the (T,G)-A--L complexed with a "carrier" protein, e.g., methylated bovine serum albumin (MBSA); (*b*) nonresponder mice immunized with MBSA-(T,G)-A--L produce approximately the same numbers of anti-(T,G)-A--L antibody-producing cells as do responder mice immunized with (T,G)-A--L or MBSA-(T,G)-A--L (utilizing a modified hemolytic plaque-forming cell assay [F. C. Grumet, unpublished observation]); (*c*) the anti-(T,G)-A--L secondary antibody response is thymus dependent, i.e., is greatly reduced in thymectomized mice (3, 4). Two important concepts are implied by the above body of data: first, responder and nonresponder mice are both capable of synthesizing anti-(T,G)-A--L antibody molecules, and, second, the immune

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‡ Present address: National Institute for Medical Research, Mill Hill, London, England.

§ Reprint requests should be addressed to Dr. Grumet at Stanford.

‖ Senior Investigator of the Arthritis Foundation.

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; CFA, complete Freund's adjuvant; *Ir-1*, *immune response-1*; MBSA, methylated bovine serum albumin; 2-Me, 2-mercaptoethanol; MEM-PM, minimum essential medium; PBS, phosphate-buffered saline; SRBC, sheep red cells; (T,G)-A--L, poly-L(Tyr, Glu)-poly-D,L-Ala--poly-L-Lys.

defect in the nonresponders appears to be a faulty or incomplete specific recognition mechanism for the antigen under discussion.

It is, therefore, not unreasonable to postulate that the *Ir-1* gene effect may be exerted at the level of a thymus-derived "T"-cell, rather than by an antibody-producing "B"-cell (5). A minimum prediction of such a postulate would be that thymectomy would convert responder mice into a nonresponder pattern of anti-(T,G)-A--L antibody formation, without affecting the antibody response pattern of the nonresponders.

Responses to many other antigens, such as bovine serum albumin (BSA) and sheep red cells (SRBC) appear to be similarly thymus dependent. For a number of these antigens, thymectomy appears to depress the secondary or IgG antibody response much more than the primary or IgM response (6–11). This effect is similar to the defective IgG formation seen in the (T,G)-A--L nonresponder mice. The present study, therefore, was undertaken to evaluate the role of thymectomy in (T,G)-A--L responder and nonresponder mice. The results showed that thymectomy blocked IgG anti-(T,G)-A--L antibody formation in response to either (T,G)-A--L or MBSA-(T,G)-A--L, but did not block the IgM response.

Materials and Methods

Mice.—Male mice of the strains C3H/HeJ ($H\text{-}2^k$), C57BL/10Sn ($H\text{-}2^b$), and CBA/J ($H\text{-}2^k$) were purchased from the Jackson Laboratory, Bar Harbor, Maine; C3H.SW/SnHz ($H\text{-}2^b$, $Ig\text{-}1^a$) mice and their immunoglobulin allotype congenic partner CWB/8 ($H\text{-}2^b$, $Ig\text{-}1^b$) were obtained from the colony of Dr. L. A. Herzenberg, Department of Genetics, Stanford University. (The latter two strains of mice are congenic with the strain C3H/DiSn [$H\text{-}2^k$, $Ig\text{-}1^a$].)

Thymectomy.—Neonatal or young adult mice were thymectomized using the suction method devised originally by Miller (12). All thymus areas of thymectomized mice were checked macroscopically for the presence of thymus remnants at the completion of the experiments.

Antigens, Immunization Procedures, and Antibody Determinations.—Previous publications from this laboratory have described the preparation of (T,G)-A--L in CFA or phosphate-buffered saline (PBS), the collection of plasma, antibody determinations using rabbit anti-mouse γ-globulin sera and (T,G)-A--L 509-^{125}I in a modified Farr assay, and 2-mercaptoethanol (2-Me) reduction of antisera (2, 13). As in the preceding paper, antibody titers are expressed as per cent antigen bound at a particular serum dilution. MBSA was purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio, and the MBSA-(T,G)-A--L complex was prepared as previously described (3).

Irradiation.—Mice were irradiated in a circular plastic box with individual compartments using a Siemens X-ray machine (Siemens Corp., Electromedical Div., Iselin, N.J.) (in the Department of Radiobiology at Stanford) operating under conditions of 250 kv, 15 ma, 0.25 mm Cu plus 1.0 mm Al and a half-value layer of 1.10 mm Cu. The dose rate was 80 rads/min with a focal skin distance of 60 cm.

Preparation of Cells.—Single cell suspensions were prepared by disrupting organs over 50-mesh stainless steel screen in minimal essential medium (MEM-PM).[2] Bone marrow plugs

[2] MEM-PM = minimal essential medium (Eagle) purchased from Grand Island Biological Co., Grand Island, N.Y. Instant tissue culture medium powder without $NaHCO_3$ was made up with $Na_2HPO_4 \cdot 12H_2O$ (0.001 M) and $MgCl_2 \cdot 6H_2O$ (0.001 M), instead of bicarbonate, in deionized water.

were flushed from femoral and tibial cavities using MEM-PM, and were disrupted by aspiration through a Pasteur pipette. Cells for counting were suspended in filtered 0.37% formaldehyde-saline solution containing 0.1 mg/ml saponin (Coulter Electronics Inc., Hialeah, Fla.) and counts were performed using a precalibrated Coulter counter, Model B, fitted with a 100 μ aperture.

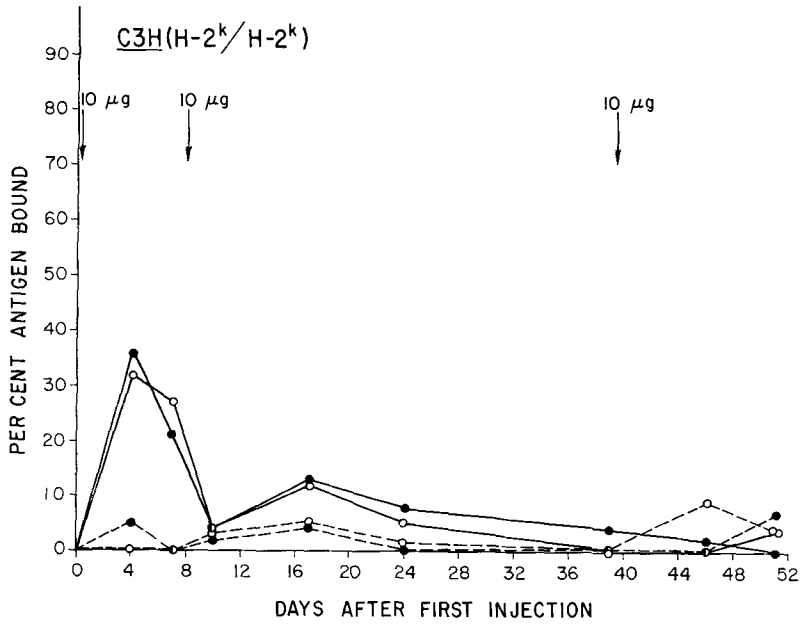
RESULTS

Influence of Adult Thymectomy Combined with Irradiation on the Primary and Secondary Response to Aqueous (T,G)-A--L.—Male mice of the C3H/HeJ and C3H.SW/SnHz strains were thymectomized or sham-thymectomized at 5–8 wk of age. 18 days later, they were X-irradiated with a dose of 750 rads and injected with 4.7 million syngeneic bone marrow cells intravenously. 28 days after irradiation, groups of three or four mice were injected intraperitoneally with 10 or 100 μg (T,G)-A--L 52 in PBS and bled at various times from 4 to 51 days later. Secondary and tertiary challenges of antigen in PBS were given on days 7 and 39. The mean plasma antibody titers are presented in Figs. 1 *a*, 1 *b*, 2 *a*, and 2 *b*. No significant differences in titers were apparent between thymectomized and sham-thymectomized mice at 4 and 7 days after the initial injection of 10 or 100 μg of (T,G)-A--L (Figs. 1 *a*–2 *b*). Both nonresponder (C3H, *H-2*^{k}) and responder (C3H.SW, *H-2*^{b}) mice reacted to the initial antigen challenge by producing early antibody which was sensitive to treatment with 2-Me and which had been previously identified in intact mice as IgM antibody (2).

Sham-thymectomized *H-2*^{b} mice (responder) reacted differently to secondary and tertiary antigen challenges than did (*a*) their thymectomized counterparts, (*b*) thymectomized *H-2*^{k} mice, and (*c*) sham-thymectomized *H-2*^{k} mice. High titer anti-(T,G)-A--L antibodies were present in sham-thymectomized responder mice after 100 μg secondary and tertiary challenges, and the 10 μg tertiary challenge. This antibody was resistant to treatment with 2-Me and was, therefore, presumptively IgG immunoglobulin. The increase in titer in these sham-thymectomized *H-2*^{b} mice is significant even though group sizes are small (e.g., at day 17 in Fig. 2 *b*, the response in three sham-thymectomized C3H.SW mice was 48 $\pm$7% compared with 7 $\pm$2% in three thymectomized C3H.SW mice [$P = 0.05$]). Sham-thymectomized *H-2*^{k} mice not only failed to produce 2-Me-resistant antibody, but they also failed to produce another pulse of 2-Me-susceptible antibody.

Influence of Adult Thymectomy Combined with Irradiation or Neonatal Thymectomy on the Secondary Response to (T,G)-A--L in Adjuvant.—McDevitt and Tyan have demonstrated that antibody responses to (T,G)-A--L are markedly reduced in thymectomized syngeneic radiation chimeras of the "responder" strain C57BL/6 (*H-2*^{b}) (4). The data presented in Table I confirm and extend this observation. Neonatally thymectomized CWB/8 (*H-2*^{b}) mice, unlike neonatally sham-thymectomized CWB/8 mice, respond feebly to aqueous booster injections after priming with (T,G)-A--L in Freund's adjuvant (experiment 1).

A



B

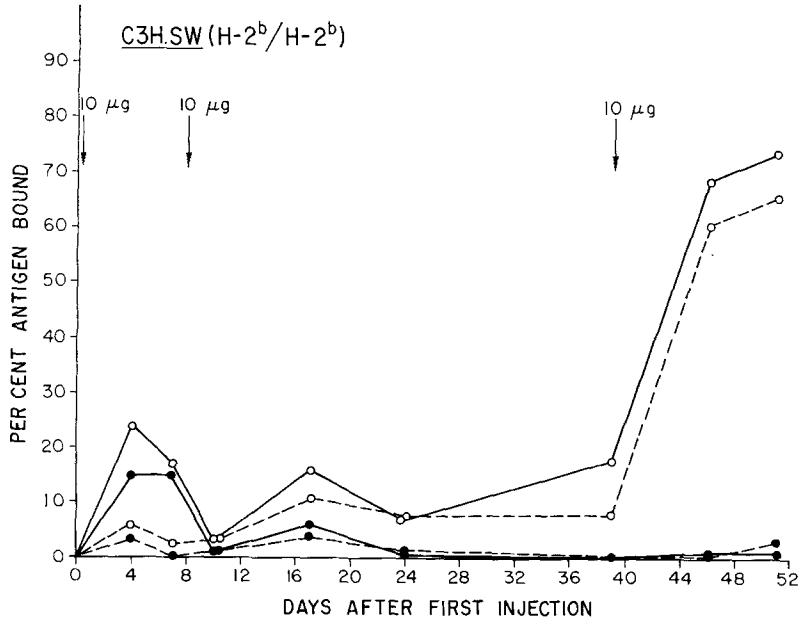


FIG. 1. The response of adult thymectomized or sham-thymectomized mice to 10 μg of aqueous (T,G)-A--L given intraperitoneally on days 0, 8, and 39: (*a*) C3H mice; (*b*) C3H.SW mice. Sham-thymectomized total antibody, —○—; sham-thymectomized 2-Me-resistant antibody, ---○---; thymectomized total antibody, —●—; thymectomized 2-Me-resistant antibody, ---●---.

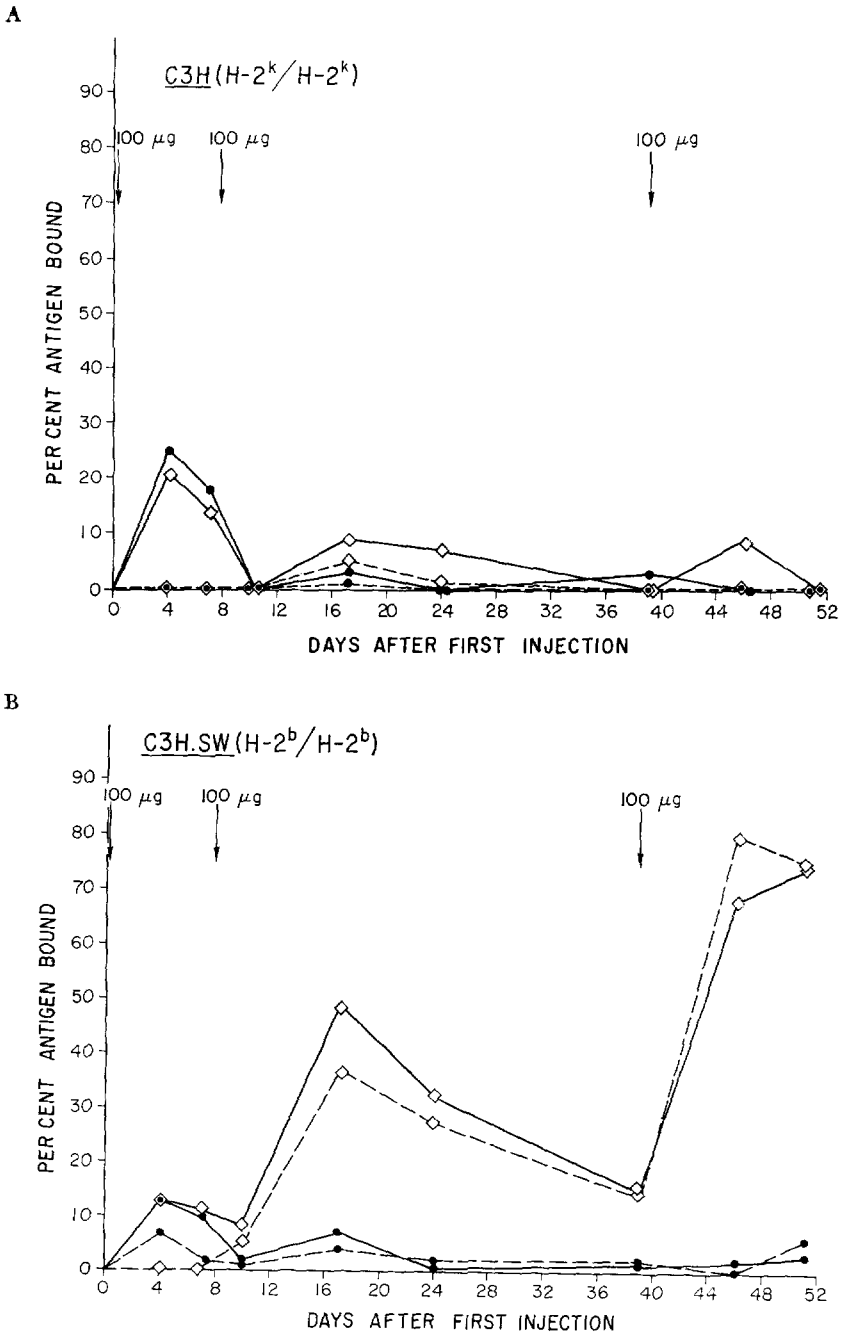


FIG. 2. The response of adult thymectomized or sham-thymectomized mice to 100 μg of aqueous (T,G)-A--L given intraperitoneally on days 0, 8, and 39; (*a*) C3H mice; (*b*) C3H.SW mice. Sham-thymectomized total antibody, —○—; sham-thymectomized 2-Me-resistant antibody, ---○---; thymectomized total antibody, —●—; thymectomized 2-Me-resistant antibody, ---●---.

A similar effect of thymectomy was noticed in adult thymectomized, bone marrow–protected mice of the C57BL/10Sn ($H\text{-}2^b$) strain injected with antigen according to a similar regimen (experiment 2).

In a third experiment, CBA/J ($H\text{-}2^k$, nonresponder) mice produced high titer antibody against (T,G)-A--L provided that the primary (in Freund's adjuvant) and secondary challenges consisted of MBSA-(T,G)-A--L. The data in experiment 3 indicate that adult thymectomy combined with irradiation prevents the conversion, by (T,G)-A--L complexed with MBSA, of nonresponder CBA mice to responder status.

TABLE I

Influence of Thymectomy on the Secondary Response to (T, G)-A--L in Adjuvant

Experiment No.*	Operation	Strain and H-2 type	Sex	Age‡	Antigen	No. of mice	Antibody responses§
				wk			
1	Neonatal thymectomy	CWB/8 ($H\text{-}2^b$)	M + F	6	(T, G)-A--L	5	5.0 ± 1.3
	Sham neonatal thymectomy	CWB/8 ($H\text{-}2^b$)	M + F	6	(T, G)-A--L	6	68.7 ± 7.8
2	Thymectomy at 5 wk, 850 R and 4×10^6 syngeneic marrow cells i.v. 3–5 wk later	C57BL/10Sn ($H\text{-}2^b$)	M	6–9	(T, G)-A--L	9	6.8 ± 4.5
	Sham-thymectomy at 5 wk 850 R and 4×10^6 syngeneic marrow cells i.v. 3–5 wk later	C57BL/10Sn ($H\text{-}2^b$)	M	6–9	(T, G)-A--L	6	55.0 ± 6.3
3	Thymectomy at 5 wk, 850 R and 4×10^6 syngeneic marrow cells i.v. 4 wk later	CBA/J ($H\text{-}2^k$)	M	10	(T, G)-A--L plus MBSA	5	6.9 ± 2.7
	Sham-thymectomy at 5 wk 850 R and 4×10^6 syngeneic marrow cells i.v. 4 wk later	CBA/J ($H\text{-}2^k$)	M	10	(T, G)-A--L plus MBSA	4	64.6 ± 4.8

* In experiment 1, mice were injected with 10 μg (T, G)-A--L 509 in CFA to footpads and i.p., and 18 days later with 10 μg aqueous (T,G)-A--L 509 to footpads and i.p. Antibody titers were determined on sera obtained 10 days after the booster using labeled (T, G)-A--L at 0.01 μg/ml and a serum dilution of 1/250. Mice were injected two times more at 3 week intervals with aqueous (T, G)-A--L and titers (at 8 days after the second boost and 6 days after the third boost) did not differ significantly from those above. In experiment 2 mice were injected with 10 μg (T, G)-A--L 509 in CFA to footpads and i.p., and 21 days later with 10 μg aqueous (T, G)-A--L 509 to footpads and i.p. Titers were determined 10 days later using labeled (T, G)-A--L at 0.05 μg/ml and a serum dilution of 1/250. In experiment 3, mice were injected with 100 μg (T, G)-A--L 52 complexed with 100 μg MBSA in CFA to footpads, and 21 days later with 100 μg each in aqueous solution. Titers were determined 10 days later using labeled (T, G)-A--L at 0.01 μg/ml and a serum dilution of 1/50.

‡ Age or time after irradiation and marrow injection in the case of radiation chimeras.

§ Per cent labeled antigen bound (±SE). All differences are highly significant ($P < 0.005$).

DISCUSSION

The genetically controlled immune response to (T,G)-A--L appears to be thymus dependent on the basis of three lines of evidence. First, adult thymectomy (combined with X-irradiation and bone marrow transfusion) or neonatal thymectomy ablate the response of (high) responder *H-2*^{b} mice immunized with (T,G)-A--L in adjuvant, confirming and extending earlier reports (4). Second, adult thymectomy also ablates the otherwise high response of "nonresponder" *H-2*^{k} mice to MBSA–(T,G)-A--L in adjuvant. Third, when aqueous antigen is used for immunization, thymectomy selectively blocks the *H-2*^{b} responder mice switchover from IgM to IgG antibody formation (after secondary or tertiary antigen challenge). The primary IgM anti–(T,G)-A--L response in both responder and nonresponder mice is not a function of *H-2* type and is unaffected by thymectomy.

Hypotheses concerning the nature of the genetic control exerted by the *Ir-1* gene must now take into consideration the results reported here and those of the preceding paper, (2) as well as the recent demonstration that the binding of ^{125}I-labeled (T,G)-A--L by unimmunized spleen cells is equal in responder, nonresponder, and congenitally athymic mice (N. Warner, personal communication).

We wish to consider two mechanisms at the cellular level which could account for the available data:[3]

(*a*) Bone marrow–derived B-cells of the IgM type (B^μ) could bind and respond to antigen in the absence of any thymus-derived T-cell influence. By contrast, B-cells of the IgG type (B^γ) might bind antigen, but would require an influence of reactive or antigen-activated T-cells to be triggered into antibody production. Perhaps B^μ-cells switch to B^γ cells and this conversion of expression of immunoglobulin constant region genes in the B-cell line is mediated, directly or indirectly, by T-cells. Alternatively, proper presentation of antigen by T-cells could be required for triggering antibody production by predetermined B^γ-cells (but not B^μ-cells). The latter possibility might reflect differences in avidity and, therefore, activating efficiency of antigen binding to receptors on B^μ- and B^γ-cells.

(*b*) Thymectomy and X-irradiation may not completely purge T-cells from peripheral lymphoid organs. (Support for this supposition could be provided by a demonstration that primary and/or secondary antibody response patterns in either congenitally athymic mice, or in adoptive experiments using cell populations proven to be completely devoid of T-cells, are different from those of mice

[3] Interpretation of many aspects of the genetic control of the immune response to (T,G)-A--L relies heavily upon recent findings of T- and B-cell interactions in responses of irradiated and thymectomized mice immunized with SRBC (5). Although there is no proof that cellular events in the immune response to (T,G)-A--L are identical to those of heterologous red cells or proteins, it will be assumed that the same qualitative mechanisms pertain, and that anti-(T,G)-A--L antibody production is in the province of B- and not T-cells.

subjected to adult thymectomy, X-irradiation, and bone marrow transfusion.) The reactive T-cells, though decreased in number, could still facilitate triggering or expression of a relatively few B-cells. If B^{μ}-cells predominated, then more of these would be recruited than would B^{γ}-cells. In the presence of reduced levels of inhibitory IgG antibody, clonal expansion of the triggered B^{μ}-cells would be accelerated. Thus IgM levels might be similar in this situation and that in which larger numbers of T-cells were present. In the latter case, more B^{μ}-cells and B^{γ}-cells could be triggered, but the clonal expansion of the B^{μ}-cells would be restricted by the appearance of early IgG antibody produced by the B^{γ}-cells.

Other indirect evidence supports the contention that in both responder and nonresponder mice, the B-cell populations are equally capable of synthesizing potent anti-(T,G)-A--L antibodies, whereas the T-cells show differential reactivity to that immunogen (1, 4, 14). Nonresponder mice may thus be considered to possess a functional or cognitive lesion in their T-cell population with respect to the carrier moiety of the (T,G)-A--L immunogen. This could produce the observed diminished secondary antibody response on the part of an otherwise competent B-cell population. The functional T-cell lesion could be bypassed when the polypeptide is complexed to a new "recognizable" carrier such as MBSA; and this new immunogen thus could convert the nonresponder to a responder status. It is apparent from the data in Table I that this conversion to responder status can be reversed by thymectomy, implying that the MBSA carrier was able to circumvent the (T,G)-A--L–specific functional lesion, but not the surgical lesion in T-populations.

No IgM memory could be demonstrated in the present system. In all C3H mice and thymectomized C3H.SW mice, secondary and tertiary antigen challenges failed to elicit any further antibody response. We have not yet determined the mechanism of this tolerance or paralysis. It is possible that B-cells, in the absence of reactive T-cells, undergo an abortive response resulting in ultimate exhaustion and thus tolerance at the B-cell level. Alternatively, it is possible that the tolerance/paralysis is the result of an active process mediated by a cellular or humoral negative feedback mechanism. As in the case of intact nonresponder mice, resolution of this problem must await further studies involving cell transfers.

The minimal predictions put forth earlier, i.e. that thymectomy should convert responder mice to a nonresponder pattern of anti-(T,G)-A--L antibody formation without affecting the pattern of the nonresponder, have been fulfilled by the data presented. These results are consistent with the hypothesis that the *Ir-1* gene effect is mediated via T-cells acting during the stage of induction of IgG antibody formation.

SUMMARY

The effect of thymectomy on the genetically controlled murine immune response-1 (*Ir-1*) to the synthetic polypeptide poly-L(Tyr,Glu)-poly-D,L-Ala--

poly-L-Lys [(T,G)-A--L] was studied with both aqueous and adjuvant immunization regimens. Adult thymectomy (combined with irradiation and bone marrow transfusion) did not affect the aqueous antigen-induced (IgM) primary response of either high or low responder mice, but did ablate the (IgG) secondary or tertiary response, a response which is restricted to the high responder strains. Adult thymectomy also blocked the normal high response to (T,G)-A--L in Freund's adjuvant in high responder mice and the high response to methylated bovine serum albumin (MBSA)-(T,G)-A--L in low responder mice. Neonatal thymectomy was also effective in blocking the response to (T,G)-A--L in Freund's adjuvant in high responder mice.

These data are consistent with the concept that the *Ir-1* gene effect is mediated via thymus cell interaction with antigen and with "B"-cells during the time of induction of IgG antibody formation.

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