GENETIC CONTROL OF IMMUNE RESPONSE

The Dose of Antigen Given in Aqueous Solution is Critical in Determining Which Mouse Strain is High Responder to Poly(LTyr,LGlu)-poly(LPro)-poly(LLys)*

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The immune response to a large number of antigens, or antigenic determinants, has been shown to be under genetic control (1-4). Several control mechanisms have been investigated. The antibody response linked to the immunoglobulin allotype is expressed in the antibody-producing cells (5-9) and, at least in some cases, it may be controlled by a structural gene for the immunoglobulin variable region (10). The gene complex linked to the major histocompatibility locus controls the response to many thymus-dependent antigens (2, 11, 12). The exact function of this locus is not yet known. It has been suggested that the gene product is responsible for the recognition of the antigen by the thymus-derived T cells (13). Evidence has also been brought forward which is compatible with the possible role of the gene product in the cooperation between T and B (the bone marrow-derived) cells, or with a genetic defect at the level of the B cells only (14-17). Recent studies have led to the discovery of a linkage between the X-chromosome and the level of the immune response to several antigens (18-20), which are mostly thymus-independent. The control mechanism of the response is not known.

Synthetic polypeptides have been widely used in the study of the genetic control of the immune response (1-4, 21). Immunization with polypeptides built on multichain polyalanine (A-L) leads to a response directed predominantly towards the determinants attached to A-L such as peptides of tyrosine and glutamic acid (T,G), phenylalanine and glutamic acid (Phe,G), and histidine and glutamic acid (H,G) (22). This response is linked to the major histocompatibility locus of the mouse (H-2), and the gene complex controlling the level of the response is called Ir-1A (23).

The response to polypeptides built on multichain polyproline (Pro-L) is controlled in a more complex way. The antibody response to poly (LPro) is under the control of two separate genes. The ability to respond to the (Phe,G) determinant is regulated by the Ir-1A gene which is linked to H-2, whereas the response to the Pro-L region of the molecule is controlled by a different gene, denoted Ir-3, which is not linked to H-2 (24). The capacity to elicit immune responses to poly (LPro) is governed by at least three response genes. The response potential to the (H,G) part of the immunogen is controlled by the Ir-1A gene, a second gene (Ir-3) controls the response to Pro-L, and a

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third gene controls the immune response to determinants characterized by a combination of both the (H,G) and Pro- -L moieties. The last immune response gene(s) was found not to be linked to H-2 (25). Upon immunization with poly(tTyr,tGlu)-poly(tPro) - poly(tLys), [(T,G)-Pro--L], the specificity of the antibody elicited was found to be directed exclusively towards Pro- -L, and the ability to mount an immune response to this immunogen is governed by the Ir-3 gene (24, 26). The Ir-3 gene is not linked either to the H-2 locus, or to immunoglobulin allotypes (24), nor is it linked to the X-chromosome (personal observation). The defect in the immune response of DBA/1, low responder mice to (T,G)-Pro--L, has been shown to be expressed at the B-cell level, using the limiting dilution analysis (27) and in experiments in which a thymus factor specific to (T,G)-Pro--L was utilized (28, 29).

Studies on the genetic control of immune response towards the synthetic multichain antigens mentioned above have been carried out with animals immunized in complete Freund's adjuvant. In such studies, the differences between low and high responder strains were not reflected in the immunoglobulin class of the antibodies formed (30). On the other hand, more recent studies of Grumet (31) have demonstrated that immunization of low and high responder mice with an aqueous solution of poly(tTyr,tGlu)-poly(pAla)--poly(tLys),[(T,G)-A--L], leads to antibody formation, and that the genetic defect is expressed as inability of the low responder strain to produce IgG antibodies in a secondary response.

In the present study, the appearance of the antibody-forming cells and of serum antibody responses, after immunization with (T,G)-Pro- -L in aqueous solution were investigated. The results show that there is no difference between the low responder DBA/1 and high responder SJL strains, when they are immunized with an optimal dose (1 µg) of antigen. With a higher dose of the immunogen, the response in the high responder strain is higher than in the low responder strain, whereas a suboptimal dose induces a higher response in the low responder than in the high responder strain.

Materials and Methods

**Animals.** Mice of the inbred SJL and DBA/1 strains were obtained from the Experimental Animal Unit of The Weizmann Institute. Mice of both sexes were used at 8-16 wk of age.

**Antigens and Immunization Procedures.** Poly(tTyr,tGlu)-poly(tPro)-poly(tLys), designated (T,G)-Pro- -L, 935, was used as the immunogen in this study. Poly(tPro)--poly(tLys),Pro- -L, 927, and poly(tTyr,tGlu)-poly(pAla)--poly(tLys),[(T,G)-A--L], 1383, were used in the inhibition of plaque formation. The synthesis and characterization of these polypeptides have been described earlier (32, 33).

For the plaque-forming cell (PFC) assay, mice were immunized intraperitoneally with different doses of the antigen in 0.2 ml of phosphate-buffered saline (PBS, 0.15 M NaCl-0.01 M phosphate buffer, pH 7.0). For the secondary response a booster injection was given 3 wk after the first immunization, and for the tertiary response 6 wk after the second injection of the antigen. Antibodies were titrated in the sera of animals immunized into the hind foot pads with different doses of antigen in 0.06 ml of PBS.

**Hemolytic Plaque-Forming Cell Assay.** Plaque-forming cells were detected by the method of Jerne et al. (34), using (T,G)-Pro- -L-coated sheep red blood cells (SRBC), as described earlier (35). Indirect PFC were calculated by subtracting the number of direct plaques developed with the aid of rabbit antismouse IgG serum which was prepared as described by Herzenberg and Herzenberg (36).

Inhibition of plaque formation was done according to Davie et al. (37). Briefly, four different concentrations of antigen (10-fold difference) were mixed with melted top agar, spleen cell
suspension, and antigen-coated SRBC immediately before plating. Percent of inhibition was calculated for each concentration of antigen.

**Antibody Titration.** Antibodies were titrated by passive microhemagglutination (38), using fresh SRBC which were coated with antigen using CrCl₂ as a coupling agent (39).

**Tolerance Induction.** In order to induce tolerance (T,G)-Pro-L was injected in low and high doses. For the low zone tolerance, mice were given 3 weekly injections of the supernate obtained after centrifugation of the antigen at 15,000 rpm for 30 min. For the induction of the high zone tolerance, mice were given one or two injections of the antigen in either centrifuged or not centrifuged form.

## Results

**Kinetics of Antibody-Forming Cell Response to Different Doses of Antigen.** The number of direct and indirect PFC to (T,G)-Pro-L in the spleens of SJL high responder and DBA/1 low responder mice was followed during the primary, secondary, and tertiary response to different doses of antigen given in aqueous solution. The immune response to 1 μg of (T,G)-Pro-L, which is depicted in Fig. 1, was similar in both strains. The peak of direct PFC appeared 4 days after the first, second, and third injection of the antigen. In the secondary response the number of direct PFC was higher and remained at a high level longer than in the primary response. There was no significant difference in the number of direct PFC between the strains.

When mice were immunized with a higher dose, 10 μg of (T,G)-Pro-L, a difference between the strains could be shown in two ways in the primary response. In some experiments, shown in Fig. 2, both strains produced a similar high number of direct plaques in the primary response, but the peak appeared 7 days later in the low responder DBA/1 strain than in the high responder strain. In some other experiments the difference between the strains was quantitative. The high responder SJL mice produced a higher number of direct plaques than the low responder mice. In the secondary response to this dose there was no

![Fig. 1. Kinetics of the direct hemolytic PFC response to 1 μg of (T,G)-Pro-L in SJL and DBA/1 strains. Primary, secondary and tertiary responses. (O—O), SJL strain; (●—●), DBA/1 strain. Each point represents the geometric means of five mice ± SE.](image-url)
significant difference between these two strains either in the kinetics or in the number of the direct PFC.

In the immune response to 100 µg dose, which is shown in Fig. 3, both strains produced a similar low number of plaques in the primary response. In the secondary response the high responder SJL strain produced a four times higher number of direct PFC than the low responder strain.

The production of indirect PFC by SJL and DBA/1 mice in the response to (T,G)-Pro--L given in aqueous solution was also investigated. The kinetics of the secondary response to different doses of antigen is shown in Fig. 4. In the response to 1 µg dose no significant difference could be shown between the strains in the number of plaques and in the appearance of the peak of the response. The response to 10 µg dose was lower than the response to 1 µg dose. No difference was shown between the strains. In the response to 100 µg dose a quantitative

![Fig. 2. Kinetics of the direct hemolytic PFC response to 10 µg of (T,G)-Pro--L in SJL and DBA/1 strains. Primary and secondary responses. (O—O), SJL strain; (●—●), DBA/1 strain. Each point represents the geometric means of five mice ± SE.](image)

![Fig. 3. Kinetics of the direct hemolytic PFC response to 100 µg of (T,G)-Pro--L in SJL and DBA/1 strains. Primary and secondary responses. (O—O), SJL strain; (●—●), DBA/1 strain. Each point represents the geometric means of five mice ± SE.](image)
difference was shown between the strains. The high responder SJL mice produced four times more indirect plaques than DBA/1 mice.

The indirect PFC response to the optimal 1 μg dose was followed in the primary, secondary, and tertiary response. The results are summarized in Table I. Both strains produced an equal number of indirect plaques already in the primary response. The number of indirect plaques was higher in the secondary than in the primary response. In the tertiary response to 1 μg dose both strains produced an equally high number of indirect plaques, but a delay of 6 days was

![Graph](image_url)

**Fig. 4.** Kinetics of the indirect hemolytic PFC responses to 1 μg, 10 μg and 100 μg of (T,G)-Pro-L in SJL and DBA/1 strains. Secondary response, (O-O), SJL strain; (○-○), DBA/1 strain. Each point represents the geometric means of five mice ± SE.

| Table I |
|-----------------|-----------------|
| **Indirect Hemolytic Plaque-Forming Cells in the Primary, Secondary and Tertiary Response to 1 μg of (T,G)-Pro-L** |
| **Indirect plaques** | **SJL** | **DBA/1** |
| Exp. I | | |
| Primary response, day 4 | 6,010* (1.23) | 5,650 (1.24) |
| Secondary response, day 4‡ | 11,200 (1.14) | 12,800 (1.14) |
| Exp. II | | |
| Secondary response, day 4‡ | 8,790 (1.25) | 14,600 (1.23) |
| Tertiary response§ | | |
| Day 4 | 2,330 (1.58) | 440 (1.33) |
| Day 8 | 320 (1.33) | 440 (1.07) |
| Day 14 | 660 (1.58) | 2,210 (1.29) |

* Number of PFC per spleen, geometric means of five mice, standard errors are in parentheses.
‡ The second injection of antigen was given 3 wk after the first injection.
§ The third injection of antigen was given 6 wk after the second injection.
shown in the appearance of the peak of the response in the low responder DBA/1 strain.

Kinetics of the direct and indirect PFC response to a suboptimal dose of (T,G)-Pro-\textsubscript{L}, 0.02 \(\mu g\), was followed in the primary and secondary response (shown in Table II). In the primary response the low responder DBA/1 strain produced 10 times more indirect plaques than the high responder strain. Also the number of direct plaques was higher in the low responder strain. As the result was unexpected, the experiment was repeated several times and every time a 4–20 times higher number of indirect plaques was produced by the low responder DBA/1 mice. In the secondary response no difference could be shown between the strains in the number of direct or indirect plaques.

**Antibody Titers in Sera.** Mice were immunized with different doses of antigen in aqueous solution into the hind foot pads. The results of the antibody titrations by passive microhemagglutination are shown in Figs. 5–7. When mice

| Table II |
|------------------------|
| **Direct and Indirect Hemolytic Plaque-Forming Cells in the Primary and Secondary Response to 0.02 \(\mu g\) of (T,G)-Pro-\textsubscript{L}** |
| | Direct PFC | Indirect PFC |
| | SJL | DBA/1 | SJL | DBA/1 |
| Primary response, day 4 | | | | |
| Exp. I | 4,320* (1.38) | 9,920 (1.10) | 265 (1.58) | 4,985 (1.06) |
| Exp. II | 3,100 (1.16) | 6,950 (1.19) | 545 (1.14) | 2,100 (1.10) |
| Exp. III | 1,620 (1.63) | 5,520 (1.73) | 400 (1.19) | 1,945 (1.74) |
| Exp. IV | 840 (1.29) | 3,840 (1.32) | 65 (1.63) | 970 (1.10) |
| Secondary response, day 4† | | | | |
| Exp. IV | 1,290 (1.30) | 1,830 (1.21) | 196 (1.38) | 510 (1.29) |

* Number of PFC per spleen, geometric means of five mice, standard errors are in parentheses.
† The second injection of antigen was given 3 wk after the first injection.

were immunized with 1 \(\mu g\) of (T,G)-Pro-\textsubscript{L}, both high and low responder mice produced 2-mercaptoethanol (2-ME) resistant antibodies in the primary and secondary response, slightly more being produced by the low responder strain than high responder strain in the primary response. In the tertiary response the titer of 2-ME resistant antibodies decreased in the low responder strain (Fig. 5).

In the primary response to the 10 \(\mu g\) dose, shown in Fig. 6, no significant difference could be shown between the strains in the antibody titers, which were low in both strains. In the secondary and tertiary response to this dose the total antibody titers were higher in the high responder SJL mice than in DBA/1 mice. In the secondary and tertiary response 2-ME-resistant antibodies could be detected only in the high responder strain above the background level.

When mice were immunized into the foot pads with the 100 \(\mu g\) dose of (T,G)-Pro-\textsubscript{L}, (Fig. 7), the high responder mice produced higher amounts of antibodies in the secondary response than the low responder DBA/1 mice. In the tertiary response the total antibody titer increased in the high responder and decreased in the low responder strain. With this dose the low responder mice did not produce any detectable 2-ME-resistant antibodies.
Inhibition of Plaque Formation. Inhibition of the plaque formation with (T,G)-Pro--L was done on day 4 of the secondary response to the 1 μg dose, on which day a similar number of direct and indirect plaques were produced by both strains. The results are shown in Table III. Percent of direct and indirect PFC inhibited by each concentration of free antigen was calculated. There was no significant difference between the strains in the inhibition of direct or indirect plaque formation by any concentration of antigen. Indirect plaques were

![Graph of Fig. 5](image)

**Fig. 5.** Hemagglutination titers in sera of SJL and DBA/1 mice immunized with 1 μg of (T,G)-Pro--L in saline into the foot pads. (O—O), SJL, total antibody titer; (●—●), DBA/1, total antibody titer; (O—O), SJL, 2-ME resistant antibody titer; (●—●), DBA/1 2-ME resistant antibody titer. Each point represents a pool of five mice.

![Graph of Fig. 6](image)

**Fig. 6.** Hemagglutination titers in sera of SJL and DBA/1 mice immunized with 10 μg of (T,G)-Pro--L in saline into the foot pads. (O—O), SJL, total antibody titer; (●—●), DBA/1, total antibody titer; (O—O), SJL, 2-ME resistant antibody titer; (●—●), DBA/1, 2-ME resistant antibody titer. Each point represents a pool of five mice.
inhibited more readily by free antigen than direct plaques in both strains. Approximately a 10 times higher concentration of antigen was needed for the inhibition of direct plaques than for the inhibition of indirect plaques.

In the primary response to the 0.02 µg dose, the inhibition of the plaque formation was performed using (T,G)-Pro--L, Pro--L, and (T,G)-A--L as inhibitors (Table IV). This was done in order to test the possibility that, upon immunization with 0.02 µg of (T,G)-Pro--L, the DBA/1 mice would produce antibodies to the (T,G) determinant, rather than to the Pro--L region, as with the higher doses used, antibodies were produced only to Pro--L. No inhibition could be obtained with a micromolar concentration of (T,G)-A--L, whereas a 10 times lower concentration of (T,G)-Pro--L and Pro--L inhibited the hemolytic

![Hemagglutination titers in sera of SJL and DBA/1 mice immunized with 100 µg of (T,G)-Pro--L in saline into the foot pads.](image)

**TABLE III**

*Inhibition of Hemolytic Plaque-Formation with the Free Antigen in the Secondary Response to 1 µg of (T,G)-Pro--L*

| Conc. of inhibitor* | Direct plaques | Indirect plaques |
|--------------------|----------------|-----------------|
|                    | SJL            | DBA/1           | SJL            | DBA/1           |
| 10⁻¹ M             | 95.7 (±1.4)%   | 96.2 (±1.0)%    | 100            | 100             |
| 10⁻² M             | 88.1 (±3.5)%   | 95.5 (±2.5)%    | 99.3 (±0.5)    | 100             |
| 10⁻³ M             | 32.8 (±6.7)%   | 42.8 (±6.7)%    | 62.1 (±13.7)   | 70.1 (±11.9)    |
| 10⁻⁴ M             | 5.0 (±3.2)%    | 17.6 (±3.9)%    | 42.2 (±8.7)    | 40.7 (±17.2)    |

* (T,G)-Pro--L used as inhibitor.
‡ Percent of plaques inhibited with the given concentration of inhibitor, means of five mice. Standard errors are in parentheses.
plaque formation to the background level. Thus, the specificity of the antibodies was towards the Pro--L region.

**Induction of Tolerance.** For the induction of high zone tolerance, mice were injected with different doses of (T,G)-Pro--L which was not centrifuged or with 1 mg of the antigen in the centrifuged form. Booster injection, 1 μg of (T,G)-Pro--L in saline, was given three wk after the first injection of the antigen (Table V). No difference could be shown between the strains in the readiness to induce tolerance. After two injections of deaggregated (T,G)-Pro--L the PFC response was reduced to the background level in both strains. To induce the low zone tolerance, mice were injected three times a week with 1 ng of deaggregated (T,G)-Pro--L. Tolerance was obtained in both strains after seven injections of the tolerogen (Table VI).

**Discussion**

The difference in antibody titers between the high and low responder mice to (T,G)-Pro--L was originally shown by experiments in which antigen was given in complete Freund's adjuvant (24). The high responder strain produced higher

| Table IV | Inhibition of Hemolytic Plaque-Formation with Different Antigens in the Primary Response to 0.02 μg of (T,G)-Pro--L |
|----------|---------------------------------------------------------------------------------------------------------------|
| Conc. of inhibitor | Direct plaques | Indirect plaques |
| | SJL | DBA/1 | SJL | DBA/1 |
| — | 690* | 3,120 | 116 | 1,210 |
| 10^-7 M (T,G)-Pro--L | 90 | 60 | 40 | 177 |
| 10^-4 M Pro--L | 60 | 45 | 88 | 178 |
| 10^-4 M (T,G)-A--L | 855 | 2,940 | 182 | 1,110 |

* Spleens of five mice were pooled.

| Table V | Induction of High Zone Tolerance to (T,G)-Pro--L |
|----------|------------------------------------------------------------------------------------------------|
| First injection of (T,G)-Pro--L | Direct plaques |
| | SJL | DBA/1 |
| 200 μg | 19,300 (1.43)* | 25,700 (1.15) |
| 500 μg | 14,200 (1.26) | 17,400 (1.17) |
| 1 mg | 870 (1.31) | 1,110 (1.32) |
| 1 mg (centrifuged) | 750 (3.20) | 280 (1.25) |
| 1 mg + 1 mg (centrifuged)‡ | 27 (1.56) | 21 (1.29) |

* Direct PFC response to 1 μg of (T,G)-Pro--L, which was not centrifuged, given 21 days after the first injection. Geometric means of five mice, standard errors are in parentheses.
‡ Two injections of centrifuged (T,G)-Pro--L, 1 mg each, given at 7 days interval.
TABLE VI

Induction of Low Zone Tolerance to (T,G)-Pro--L

| No. of injections* | Direct plaques |
|-------------------|----------------|
|                   | SJL            | DBA/1          |
| 5                 | 9,100 (2.73)$  | 34,400 (1.08)  |
| 7                 | 295 (1.31)     | 310 (1.23)     |

* 1 ng of (T,G)-Pro--L, in a centrifuged form, given three times a week.
† Direct PFC response to 1 μg of (T,G)-Pro--L, which was not centrifuged, given 7 days after the last injection of tolerogen. Geometric means of five mice, standard errors are in parentheses.

amounts of antibodies in the secondary response than the low responder strain. The results described here show, however, that there is no difference in the antibody titers and in the numbers of direct or indirect PFC in the primary and secondary response between high and low responder mice when immunized with 1 μg, which appeared to be optimal dose, of antigen in aqueous solution (Fig. 1).

When a higher than optimal dose was used for immunization, differences between the strains could be shown. The immune response to 10 μg of (T,G)-Pro--L was lower in both strains than the response to the optimal dose. In one experiment, which is shown in Fig. 2, the difference between the strains was in the kinetics of the direct PFC, the peak of the response appearing later in the low responder DBA/1 strain than in the high responder SJL strain. A difference in the kinetics between the strains was also shown in the appearance of the peak of indirect PFC in the tertiary response to the optimal 1 μg dose (Table I).

Similar differences in the kinetics of immune response between high and low responders has been shown in the response of monkeys to the monkey herpesviruses (40). All monkeys produced antibodies to the virus, but high responders, which do not develop tumors, produced antibodies earlier than low responders, which do develop tumors. Green has also noticed a difference in the time appearance of antibodies between guinea pig strains which are responders or nonresponders to dinitrophenyl-polyllysine (41). After immunization with a complex of DNP-poly-lysine and BSA, responder guinea pigs produced antibodies 5 days earlier than nonresponders.

A difference between the strains could also be shown in another way after immunization with a high dose of antigen. In a few experiments, in which mice were immunized with 10 μg of antigen, a quantitative difference was shown between the strains in the number of direct plaques in the primary response. Also in the secondary response to the 100 μg dose the difference between the strains was quantitative (Fig. 3). The high responders produced a five times higher number of plaques than the low responders. Thus, the difference in the kinetics of the PFC response and the quantitative difference in the actual number of PFC produced by the animal may represent the same basic difference between the strains, and is possibly due to one single gene.

A quantitative difference between the strains could also be shown in the antibody titers. After immunization with 10 μg of antigen, a slight difference in the total antibody titers was shown (Fig. 6). The difference in the 2-ME-resistant antibodies was significant. In the response to 100 μg of (T,G)-Pro--L, both the total antibody titer and the 2-ME-resistant antibody titer were clearly higher in the high responder than in the low responder strain in
The difference in the antibody formation between the high and low responder mouse strains injected with 10 \( \mu \text{g} \) of (T,G)-Pro--L in complete Freund's adjuvant resembles the results reported here upon immunization with 100 \( \mu \text{g} \) of antigen in aqueous solution. This is in agreement with previous findings that the immune response to a small dose of an antigen in complete Freund's adjuvant resembles in the kinetics and titers obtained the response to a much higher dose administered in saline (42).

The immune response to a suboptimal dose of antigen was unexpected, as a higher response was obtained in the low responder strain than in the high responder strain in the primary response. In all experiments the low responder strain produced 4–20 times more indirect plaques than the high responder strain (Table II). The number of direct plaques was also higher in the low responder strain in the primary response. The secondary response was not significantly different in these two strains. That the dose may play a crucial role in defining the genetic control of an immune response to a synthetic antigen, has been shown previously by Dorf et al. (43). They found that the immune response to GAT was controlled by several genes. Besides the \( H-2 \)-linked gene(s) which determines whether a strain is a responder or a nonresponder, the non-\( H-2 \)-linked genes regulate the difference in the amounts of antibodies produced by the responder strains. An \( H-2 \)-linked genetic control in the response to a limiting dose of protein antigen has been shown before (44, 45). Recently, it has been shown that in the response to LDH-H\(_4\) mice strains of \( H-2^a \) type are high responders to all doses, mice of \( H-2^{a,b,c} \) type are responders to 100 \( \mu \text{g} \) dose but nonresponders to 10 \( \mu \text{g} \) and mice of \( H-2^a \) type are nonresponders to both doses of LDH-H\(_4\) (Falkenberg and Mozes, unpublished results). In all the above studies, lowering the dose resulted in a decreased response in the low responders whereas in this study the low responders responded better to a low dose than the high responders, and 1 \( \mu \text{g} \) was optimal for both strains.

It was of interest that in the primary and secondary response to the optimal dose both strains produced equal numbers of indirect PFC (probably IgG-producing cells). Also, after immunization with 1 \( \mu \text{g} \) of antigen into the foot pads, 2-ME-resistant (probably IgG) antibodies were found in both strains in the primary and secondary response. These results are different from those reported by Grumet (31) for (T,G)-A--L, an antigen to which the immune response is \( H-2 \) linked. The low responders to (T,G)-A--L could not produce IgG antibodies in the primary and secondary response by similar immunization. Apparently, the mechanism of the low responsiveness to these two antigens is different. In this context it is pertinent to point out the importance of macrophages in the response of high and low responder mice to (T,G)-Pro--L. The stimulation of peritoneal macrophages increased the response in the low responder strain to the level of the high responder strain, whereas the stimulation had no effect on the high responder strain (46). Similarly, the injection of poly (A)·poly(U) corrected the response to (T,G)-Pro--L in the low responder strain, but it had no effect on the high responder strain (47). In contradiction, poly(A)·poly(U) (47) and macrophages (personal observation) do not affect the difference in the response to (T,G)-A--L by the high and low responder strains.

There are several possibilities to explain the difference seen between the strains after immunization with a high dose of antigen. Such a dose could have a direct suppressive effect on the antibody-producing cells or their precursors via the
antigen-specific receptors. The specificity and affinity of such receptors have been shown in some cases to be identical with the secreted antibodies (48). Thus, a more pronounced suppression found in the low responder strain would be due to a higher avidity of the antigen specific receptors on B cells in the low responder strain. This possibility was tested by inhibition of plaque formation by the free antigen. The percent of cells inhibited by each concentration of antigen was similar in both strains, suggesting that they have similar populations of high and low avidity antibody-producing cells.

Another possible explanation for the difference between the strains would be a qualitative difference of antigen-specific receptors on T cells. (T,G)-Pro- -L, used as antigen, is thymus-dependent (3). A population of T cells with higher affinity receptors for antigen might be triggered for antibody production as well as for tolerance with a lower dose of antigen than another population having lower affinity receptors, even if the antigen-specific receptors on B cells were identical in the two strains. Thus, a higher response shown in the low responder DBA/1 strain to suboptimal dose of antigen might be explained by a higher affinity of the T-cell receptors to (T,G)-Pro- -L in this strain. The high dose, which suppressed the response more clearly in the low responder DBA/1 strain might do so by inducing tolerance easier in this strain. To test this possibility, the low zone tolerance, which has been shown to involve T cells only, as well as the high zone tolerance, in which both T and B cells are tolerant (49), were induced in both strains. No difference could be shown between the strains in the induction of either high zone or low zone tolerance. These experiments suggest that the lower response in the low responder DBA/1 strain to a higher than optimal dose of antigen is not due to induction of tolerance more readily in this strain. As the mechanism of tolerance induction is not known, no conclusion, however, can be drawn concerning the T-cell receptor on the basis of this experiment. However, the limiting dilution experiments (27) as well as the experiments in which a T-cell factor specific to (T,G)-Pro- -L could be produced similarly in both high responder SJL and low responder DBA/1 strains (29) indicate that the difference between the strains in the response to this antigen is not due to a different quality of antigen specific T-cell receptors but is expressed in B cells.

Further, the higher response obtained in the low responder than high responder strain after immunization with a low dose of antigen could be due to different specificity of antibodies produced. Although both SJL and DBA/1 strains are low responders to (T,G) determinant, when immunized with either (T,G)-Pro- -L or (T,G)-A- -L in Freund’s adjuvant, it was of interest to test whether the specificity of antibodies in the response to 0.02 μg of antigen given in aqueous solution was really anti-Pro- -L and not directed towards the (T,G) part of the antigen. As no inhibition could be obtained in the presence of (T,G)-A- -L, whereas (T,G)Pro- -L and Pro- -L inhibited similarly the plaque formation, it is concluded that the specificity of the antibodies was towards the Pro- -L region of the antigen (Table IV).

The results of the present work suggest that the difference between SJL and DBA/1 strains in the response to (T,G)-Pro- -L, which is controlled by the Ir-3 gene, is not qualitative but quantitative. After immunization with the optimal dose of antigen, there is equal stimulation (and probably also equal suppression)
in both strains. After immunization with a higher than optimal dose of antigen the suppression is more pronounced in the low responder strain, whereas with a suboptimal dose the stimulatory effect is more clear in this strain. Several explanations could be given to this phenomenon, and they all involve a regulation between stimulation and suppression. Suppressor T cells have been described recently in many reports (e.g. 50-52). The first explanation is that the ratio between stimulating and suppressing T cells would be different in these two strains after injection of different doses of antigen. The second explanation is that the amount of stimulating and suppressing factors produced by equal numbers of cells in these strains would be different. The third and the most appealing possibility is that the sensitivity of B cells to the stimulating and suppressing effect of T cells would be different in these two strains in the response to (T,G)-Pro- -L. Further experiments are currently under investigation in this laboratory to test these possibilities.

Summary
Antibody response to different doses of (T,G)-Pro- -L, given in aqueous solution, was investigated in the high responder SJL and low responder DBA/1 strains by measuring hemolytic plaque-forming cells (PFC) in the spleens as well as hemagglutination titers in the sera. The gene responsible for the difference between the two strains in the response to this antigen, given in complete Freund's adjuvant, has been previously denoted Ir-3. This gene is not linked to the major histocompatibility locus. In the response to the optimal dose (1 µg) of antigen, no difference could be shown between the strains. The peak of the response and the numbers of direct and indirect PFC were similar in both strains in the primary and secondary response. After injection of higher doses (10-100 µg) of antigen, both the direct and indirect PFC responses were lower in the low responder than in the high responder strain. Moreover, the peak of the response occurred earlier in the high responder strain in the primary response to the 10 µg dose of antigen. After administration of a suboptimal dose (0.02 µg) of antigen, the low responder strain produced in the primary response 4-20 times more indirect plaques than the high responder strain. Also the number of direct plaques was higher in the low responder than in the high responder strain. The serum antibody responses to the optimal and higher doses of antigen were parallel to the PFC responses.

From inhibition of PFC with free antigen, it was concluded that a similar proportion of cells was producing high and low affinity antibodies to (T,G)-Pro- -L in both strains. High and low zone tolerance could be induced in the two strains with (T,G)-Pro- -L, but no difference could be shown between the strains. It is suggested that the Ir-3 gene plays a role in the regulation of the balance between stimulation and suppression according to the dose of antigen given.

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