The Journal of Immunology

RESEARCH ARTICLE | JUNE 01 1980

Genetic control of the immune response to ferredoxin: linkage and mapping of T cell proliferation and antibody production genes to the MHC of mice.

FREE

L K Sikora; ... et. al

J Immunol (1980) 124 (6): 2615–2619.
https://doi.org/10.4049/jimmunol.124.6.2615

0022-1767/80/1246-2615 $02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 124, No. 6, June 1980
Printed in U.S.A.

GENETIC CONTROL OF THE IMMUNE RESPONSE TO FERREDOXIN: LINKAGE AND MAPPING OF T CELL PROLIFERATION AND ANTIBODY PRODUCTION GENES TO THE MHC OF MICE

LYDIA K. J. SIKORA AND JULIA G. LEVY

From the Department of Microbiology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5

The genetics of the immune response in the mouse were studied by using the antigenically simple, stable, naturally occurring protein ferredoxin (Fd) from *Clostridium pasteurianum*. The immune status of mice primed and boosted with Fd was assessed by using two parameters of immunity: T cell proliferation and serum antibody production with the ELISA method. In both assay systems, the response has been shown to be H-2 linked: *k*, *b*, and *s* haplotypes respond to Fd, and H-2^d mice are nonresponders. It is apparent that different immunoregulatory events modulate the response in the responder strains; these factors become evident in the recombinant analysis of the response and to date an immunoregulatory gene(s) has been mapped to at least the K/I-A subregions. F_1 analysis demonstrated a gene dose-dependent response of the strains studied.

Early studies on the genetic control of the immune response in the mouse have employed a variety of synthetic random copolymers of L-α-amino acids (1-6), branched multi-chain amino acid copolymers (7), as well as synthetic polymers of defined geometry (8, 9). Control of the immune response to these antigens has been clearly linked to the major histocompatibility complex (MHC)[1] of the mouse (H-2) and mapped to subregion for certain antigens (4, 5, 8); however, for technical reasons (suggested in 10) it has been difficult to establish the precise subregion(s) responsible for the immune response to a majority of the remaining antigens. More recently, naturally occurring proteins such as staphylococcal nuclease (11, 12), insulin (13, 14), lysozyme (15), pigeon cytochrome c (16), lactate dehydrogenase H_B (17), and myoglobin (18, 19) have proven to be more practical and reliable for the study of I-region genetics.

A molecule that lends itself well to this type of study is ferredoxin (Fd)[1] from *Clostridium pasteurianum*. It is 55 amino acids in length with a m.w. of about 6,000. Extensive studies in this laboratory by using chemically modified Fd and synthesized fragments of Fd have determined that there are two major antigenic determinants: the amino terminal heptapeptide and the carboxyterminal pentapeptide; the cysteine-rich central portion is not implicated in measurable immunologic activity; the results are summarized elsewhere (20). The antigenic character, the available synthetic probes, and the fact that it is a stable biologic molecule clearly demonstrate some of the advantages of Fd over the multi-determinant proteins used to date. The recent development of sensitive assays for T cell proliferation (21) and serum antibody (22) have permitted the study of the genetics of the immune response to Fd. This report deals with the genetic linkage of both T cell proliferation and antibody production to H-2 and the mapping of the response within the I region. The existence of absolute responders and nonresponders makes this small 'two determinant' molecule very amenable to precise dissection of immunoregulatory events.

MATERIALS AND METHODS

Mice. B10.S, B10.S(9R), and B10.HTT breeding pairs were obtained from Dr. D. C. Shreffler, Department of Human Genetics, University of Michigan, Ann Arbor, Michigan. Female NZB mice were a generous gift from Dr. A. Steinberg, Arthritis and Rheumatism Branch, National Institutes of Health, Bethesda, Maryland. B10.A(2R), B10.A(3R), and B10.A(4R) mice were bred by Dr. H.-S. Teh, U. B. C.; B10.BR, C3H, CBA, B10.D2, DBA/2, BALB/c, C57BL/10, SJL, and B10.A(5R) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine, either as adult animals or as sibling mating pairs. F_1's and all the other mice were bred in our animal facility. Mice of both sexes were used at 2 to 6 months of age.

Antigen. Fd was isolated and purified from *Clostridium pasteurianum* as described originally by Mortenson (23) and Tanaka *et al.* (24); purity was assessed as described previously (25).

Immunization. Mice were immunized subcutaneously in both flanks with a total of 0.2 ml containing 50 μg Fd in saline emulsified 1:1 in Freund's complete adjuvant (FCA) (Difco). Four or more weeks later, they were boosted in the identical manner. Seven days after the secondary immunization the mice were sacrificed by cervical dislocation, the much enlarged inguinal lymph nodes were used for the proliferation assay, and the sera were stored individually at −20°C. Fd 'non-immune' control animals were immunized with saline in FCA (1:1). Each strain was tested with an average of 12 mice (minimum of six); the experiments were performed at least two times with no significant differences between experiments ($p < 0.005$).

Antibody assay. The individual sera of Fd immune and nonimmune mice were assayed by using the solid phase enzyme-linked immunosorbent assay (ELISA) (22), which was carried out as described previously (26); the plates were coated over-

Received for publication December 6, 1979.
Accepted for publication February 28, 1980.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Abbreviations used in this paper: Fd, ferredoxin; ELISA, enzyme-linked immunosorbent assay; PPD, purified protein derivative; MHC, major histocompatibility complex; GLϕ, terpolymer (Glu, Lys, Phe); GAT, terpolymer (Glu, Ala, Tyr).

night with 0.2 ml per well of Fd at 1 μg/ml; the individual sera were tested routinely at 1:100; the developing antiserum was alkaline phosphatase-linked rabbit anti-mouse κ-chain Ig. The tests were run in triplicate, and the color was correlated to the amount Ig present on the plate with the standardizing combined ELISA-radioimmunoassay.

ELISA-radioimmunoassay. Anti-Fd antibody was purified by Michael Weaver in this laboratory by using Fd coupled to thiopropyl-Sepharose 6B (Pharmacia) immunoadsorbent with procedures described in the Pharmacia manual. Antibody from Fd immune B10.BR mice was eluted with 0.1 M glycine buffer pH 2.3 and neutralized immediately with 2.0 M Tris (pH 7.2). The purified antibody was assayed by ELISA before iodination with ^{125}I by the method of Greenwood *et al.* (27). The resulting specific activity was 2.27×10^7 cpm/mg Ig. The assay was set up as described previously (26); the ELISA was carried out, then read for absorbance on a Titertek Multiskan (Flow); the individual empty wells were then cut out and counted in a gamma counter (Beckman Biogamma). The amount of specific Ig adhering to the plate was calculated from its specific activity and correlated with the color development.

T lymphocyte proliferation assay. A modification of the method described by Lee and co-workers (21) was used for individual animals: inguinal lymph nodes were teased apart to a single cell suspension, washed twice, resuspended in RPMI 1640 containing 3.5 g per l $NaHCO_2$ and 50 mg per l gentamycin, and counted by using trypan blue exclusion. 1.6×10^6 cells in 150 μl medium were dispensed per well into 96-well Linbro microtiter plates (IS-FB-96TC); 0.05 ml of 50% human serum was added to reach a final concentration of 10% (a pool of human sera was collected, inactivated at 56°C for 30 min, and stored in aliquots at −70°C). Fd was added to a final concentration of 50 μg/ml. The animals were tested individually with and without Fd; each test was run in triplicate. Incubation was at 37°C in a humidified atmosphere and 6% CO_2. After 4 days, the cultures were pulsed for 22 to 24 hr with 1 μCi ^3H-met-thymidine (NEN) in 50 μl medium and harvested onto glass fiber filters (Whatman 934 AH), washed with distilled water and methanol, dried, and counted in 2 ml toluene omnifluor (Syndell, Vancouver) scintillation fluid in a Unilux II scintillation counter (Nuclear Chicago). The stimulation index for each animal was computed as:

$$\frac{\text{Average cpm with Fd}}{\text{Average cpm without Fd}}$$

RESULTS

Lymphoproliferative assay. The T cell proliferation assay described by Lee *et al.* (21) was used to establish the immune status of mice from four H-2 haplotypes and was performed on individual animals. It was found that the optimal conditions for the response to Fd were 1.6×10^6 cells/well and Fd at 50 μg/ml final concentration; the cultures were also tested at these cell and antigen concentrations with purified protein derivative (PPD), and although these are not the optimal conditions for this antigen, the stimulation index (cpm with Ag/cpm without Ag) was usually between 5 and 15 for PPD and was an internal control used to indicate that the assay was working, which is especially important in assessing nonresponsiveness (data not shown). The results of the survey are in Figure 1 and clearly demonstrate that the proliferation of lymph node cells is linked to H-2: H-2^k and H-2^s mice are responders (SJLs have abnormally high proliferation), H-2^d are nonresponders, and H-2^b show low but significant stimulation. In all but the NZB mice, which show background stimulation (cpm without Ag) over 25,000 cpm, the background stimulation was usually between 100 and 1000 cpm.

Calibration of ELISA. In order to assess the amount of specific anti-Fd antibody present in the serum of the mice tested in the proliferation assay, the ELISA was calibrated by using a radioimmunoassay. The ELISA was carried out by using iodinated immunoadsorbent-purified anti-Fd Ig from immune B10.BR mice. After reading the ELISA results for ab-

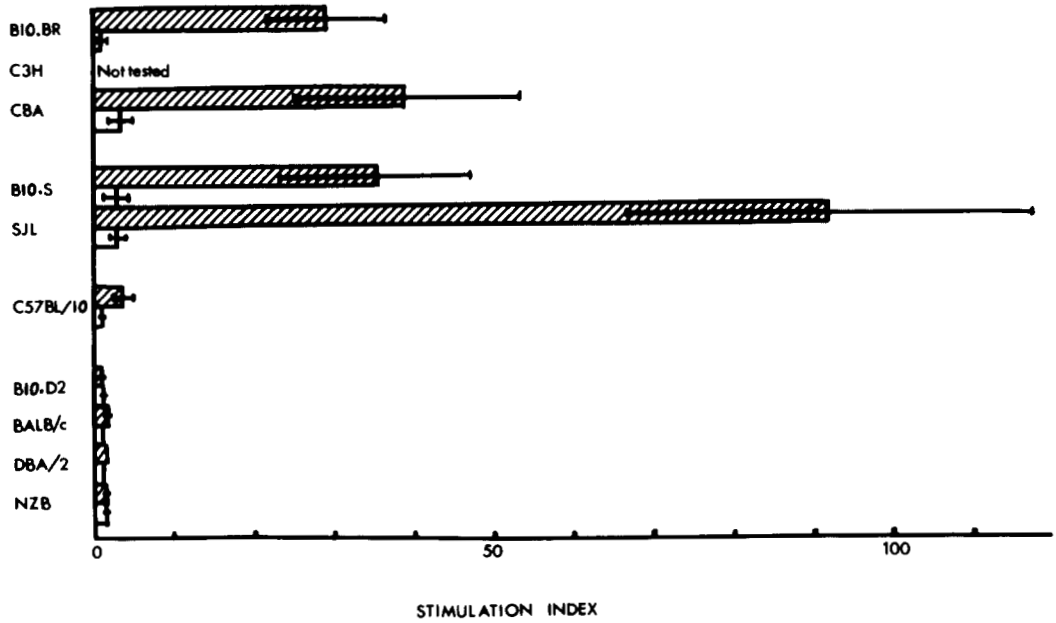

Figure 1. T-cell proliferation assay of four major haplotypes. The draining lymph node cells of mice which had received 7 days previously a secondary immunization of 50 μg Fd in FCA subcutaneously in both flanks were suspended at 1.6×10^6 cells/well in RPMI 1640 + 3.5 g/l $NaHCO_3$ with 10% heat-inactivated human serum and Fd at a final concentration of 50 μg/ml. Incubation was for 5 days at 37°C, 6% CO_2 with a 22- to 24-hr ^3H-met-thymidine pulse. The stimulation index is the ratio of events with and without Fd ± S.E.M. The hatched bars are the immune animals, the open bars are the nonimmune controls.

sorbance, the empty wells were cut out and counted, their radioactivity being translated to ng Ig by using the specific activity of the original sample. This double assay permits the correlation between the enzymatic color development and the amount of Ig adhering to the plate. The results are plotted as ng Ig per well against the absorbance at 405 nm obtained from the colorimetric enzymatic ELISA assay (for 0.25 ml), and the resulting curve, for the dilution of developing antibody used in this system, is linear between 0.3 and 1.6 units of absorbance at 405 nm and is shown in Figure 2. Sera were tested routinely at 1:100, and those that fell on the nonlinear part of the graph (i.e., below 0.3 units) were retested at a lower dilution (up to 1:20). Dilutions of less than 1:20 were not used due to nonspecific background. The accurate lower detection limit at 0.3 units is 8.0 ng Ig in 0.25 ml reaction mixture.

Antibody response to Fd. The amount of anti-Fd Ig (the developing serum is anti-κ) produced in the mice tested above for lymph node cell proliferation was quantitated by using the ELISA assay, and the results are shown in Figure 3. Individual sera were tested at 1:100. Values for nonimmune animals are not shown, since the levels of natural (noninduced) anti-Fd Ab were between 0 and 1.00 μg/ml serum in all cases (specific anti-Fd Ig can be measured accurately down to 60 ng/ml serum). MHC linkage is again clearly demonstrated; H-2^k animals are high antibody producers (9 to 16 μg/ml anti-Fd Ab), H-2^s and H-2^b are intermediate producers (2 to 9 μg/ml), and H-2^d are again nonresponders (0 to 2 μg/ml) as measured 7 days after the secondary immunization with Fd.

Antibody response of recombinant strains. The genetics of the net immunoregulatory events were studied by looking at the antibody response to a secondary stimulus with Fd in recombinant strains on the B10 background. The results of the ELISA assay are shown in Figure 4. Again, levels of natural anti-Fd antibody in nonimmune controls were between 0 and 1.00 μg/ml serum, and the data are not shown. On the basis of these results, it can be said that the B10.A(2R) and B10.A(4R)

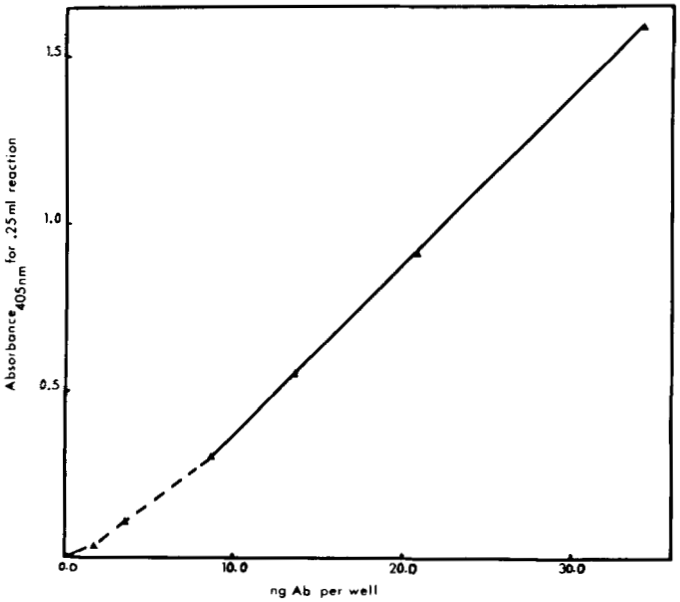

Figure 2. ELISA-Radioimmunoassay calibration curve. Fd was adhered to an ELISA polystyrene plate at 1 μg/ml. Specific Ig was labeled with ^{125}I. The enzymatic color development with alkaline phosphatase-linked rabbit anti-mouse-kappa-chain Ig was measured for absorbance at 405 nm and compared to the counts per cell. The results were related to ng Ig from the specific activity of 2.27×10^7 cpm/mg Ig. The dotted line represents the nonlinear part of the graph.

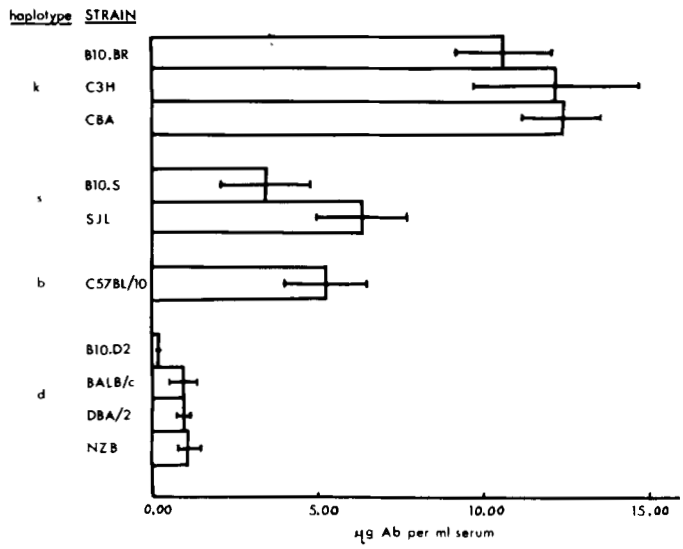

Figure 3. Antibody response of mice tested for proliferation in Figure 1. Mice were primed and boosted with 50 μg Fd in FCA subcutaneously in both flanks. Seven days after the secondary immunization, the ELISA assay was performed on a 1:100 dilution of antiserum. The absorbance at 405 nm was related to ng Ig by using Figure 2. Each result represents 12 mice on the average ± S.E.M. Nonimmune sera were between 0 and 1 μg/ml serum and are not shown.

recombinants maintain the high responder status of B10.BR; therefore, the subregion K/I-A seems to contain adequate genes that confer the high responsiveness in the '*k*' allele. Also, the B10.S(9R) recombinant has a response identical to the B10.S strain, further locating the control to the K/I-A/I-B subregion for the '*s*' allele. The B10.A(3R) and B10.A(5R) recombinants exhibit an intermediate-type responsiveness that is not significantly lower than the parental C57BL/10 response. The B10.HTT strain is considered nonresponsive (the response is significantly lower than B10.S, $p < 0.015$), and at this time there is no information on the immunoregulatory mechanisms in this strain, but multiple-region control may be implicated.

Antibody production in F_1 animals. Analysis of the anti-Fd antibody response was further studied in a limited number of available F_1 strains, and the results are shown in Figure 5. Again, the animals were tested 7 days after the secondary immunization, and the nonimmune controls are not shown. The results clearly demonstrate that the magnitude of the antibody response is gene dose dependent in all cases: the high response of B10.BR is considerably lower in F_1's with a nonresponder parent (B10.D2 and B10.HTT) and proportionally reduced with an intermediate responder type parent (B10.S). F_1's between intermediate responders and nonresponders give a barely detectable response. F_1's between B10.D2 and B10.HTT were not available at the time of testing, but future studies ought to determine whether complementation exists between these two nonresponding strains.

DISCUSSION

Previous studies in this laboratory (20) have extensively characterized the immunologic properties of Fd from *Clostridium pasteurianum*; it was determined that Fd has two major antigenic determinants and that it is an immunogenic molecule in mice, rabbits, and guinea pigs. Its lack of antigenic complexity and the fact that it is a stable biologic molecule makes it a clean probe for many genetic, molecular, and cellular studies. In this work, we have shown that the control of the immune response to Fd, both at the cellular and humoral levels, is indeed linked

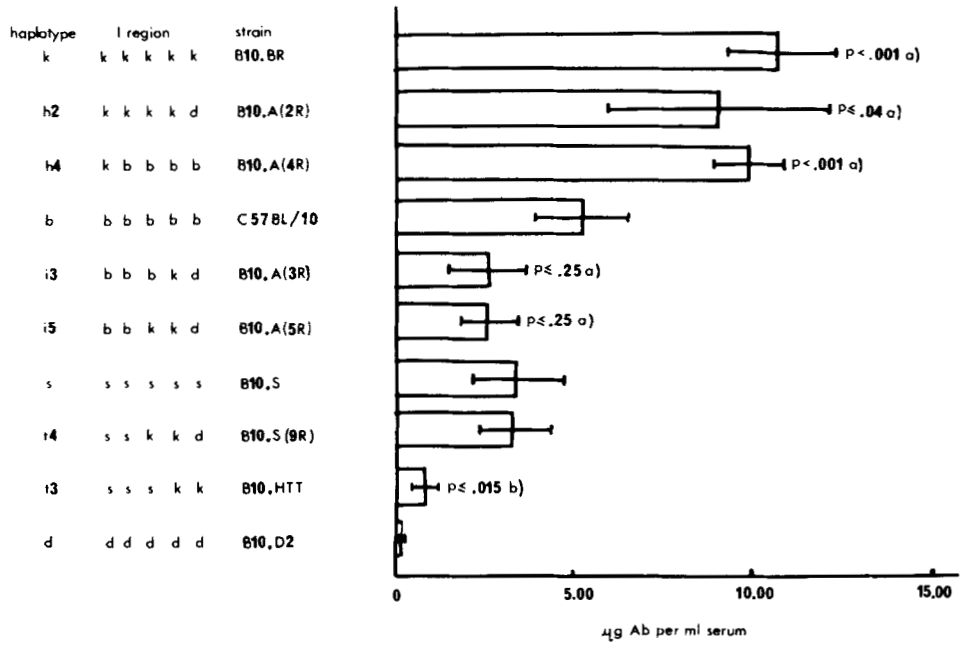

Figure 4. Antibody response of recombinant mice. I region alleles of I-A, I-B, I-J, I-E, and I-C are shown. The tests were performed under conditions described in Figure 3. Responses of B10.BR, B10.S, C57BL/10, and B10.D2 are shown for comparison. a) Responses compared by Student's *t*-test to C57BL/10. b) Responses compared by Student's *t*-test to B10.S. For all the recombinant strains the allele of the K-end genes is the same as I-A and the alleles of D-end genes is the same as I-C with the exception of B10.A(2R) which is b.

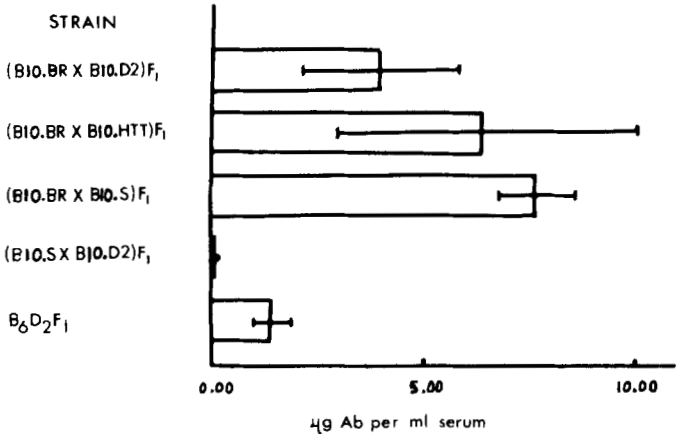

Figure 5. Antibody response of F_1 mice. The tests were performed under conditions described in Figure 3.

to H-2 and is tentatively mapped to at least the I-A subregion based on the antibody response.

The detection of the anti-Fd response required very sensitive assay systems due to the small size of the antigen. In absolute terms of responder *vs* nonresponder, we found fairly good correlation between the two assays used (ELISA and T cell proliferation (21)); this type of correlation was shown in a number of other systems, for example with the terpolymer GLϕ (Glu, Lys, Phe) (5, 6), staphylococcal nuclease (11, 12), insulin (13, 14), myoglobin (18, 19), and a number of synthetic polypeptides (28). Comparing the responses of most of the strains (NZB and SJL mice are known to have immunoregulatory defects), MHC linkage is clear; in the analysis of MHC-linked immunoregulatory events, the comparisons were made by using only the congenic lines on the B10 background and the ELISA assay. It is felt that the discrepancies between the two assay systems (T cell proliferation, Fig. 1, and antibody production, Fig. 3) may be explained by the fact that the assays are measuring different aspects of the immune response, which may be under different net immunoregulatory control. B10.BR and B10.S mice proliferate to the same extent to *in vitro* stimulus, whereas at the level of antibody production one may speculate that a negatively modulating event decreases net antibody production in B10.S mice with respect to the H-2^k response. Similarly, C57BL/10, which proliferate very poorly *in vitro* with respect to B10.S, produce similar levels of antibody as B10.S, indicating a relative positive modulation. The H-2^d animals are uniformly nonresponsive in both assays. Since our developing antiserum detects both IgM and IgG in the ELISA, it does permit us to make a prediction as to the nature of the genetic lesion in nonresponder animals: we define nonresponsiveness as a concomitant lack of IgM and IgG production as well as a lack of T cell proliferation, and this distinguishes the Fd system from the (T,G)-A-L (poly(Tyr,Glu)-poly D,L-Ala poly L-Lys) response as described by Mitchell *et al.* (29), where the genetic defect lies in the switch from IgM to IgG production, whereby all strains make primary IgM but nonresponders fail to make secondary IgG. Our system can be most readily compared in this respect with the responses of GLϕ, where nonresponders fail to make IgM and IgG, and it has been suggested that the defect in GLϕ nonresponder mice is in an early event affecting primary IgM production (30).

In the recombinant analysis of the antibody response, both B10.A(2R) and B10.A(4R) strains exhibit the high responder status of B10.BR mice, thus localizing the responsible gene(s) to the K/I-A subregion. This response is significantly higher ($p < 0.001$ for B10.A(4R) and $p < 0.04$ for B10.A(2R)) than the intermediate response of C57BL/10. Therefore, Fd tentatively joins a number of other antigens in having at least one immunoregulatory gene in I-A (e.g., the terpolymers GAT (Glu, Ala, Tyr) (4) and GLϕ (5), insulin (13), myoglobin (18), and cytochrome c (16)). No other subregion is implicated at this time in the control of the response in H-2^k mice to Fd. The fact that the B10.S(9R) mice produce identical amounts of antibody as B10.S mice further suggests that some control of the B10.S response is also located in the K/I-A/I-B subregions; however, B10.HTT mice provide an unexpected result, since they possess the intermediate-type K/I-A/I-B region (i.e., "*s*"), yet they are nonresponders or, at best, very low responders in antibody production (this response is significantly lower, $p < 0.015$, than B10.S) and proliferation (data not shown). These mice were retested on five separate occasions (total of 25 mice) with identical results. A similar type of incongruity was found in the lactate dehydrogenase H_B system with B10.HTT (17); however,

the response was rescued by complementation in F_1's. At this time, the (B10.HTT × B10.D2) F_1's are not available for a complementation test. Future studies will look into the mechanism of the B10.HTT nonresponsiveness (in parallel to B10.D2 nonresponsiveness). At this time, we can only conclude that the K/I-A subregion of *k*, *b*, and *s* haplotypes contains the genes conferring responsiveness to Fd, and speculate that genes in other subregions may modulate the response in $I\text{-}A^b$, and $I\text{-}A^s$. The F_1 analysis suggests that there is a gene dose effect in the magnitude of the response to Fd similar to the findings in other systems (31, 10). Basically, the presence of a nonresponder parent with B10.BR more than halves the response, the presence of an intermediate-responder parent proportionately reduces the B10.BR response, and in F_1's between nonresponders and intermediate responders, it is greatly diminished.

Further studies in this system will include analysis of immunoregulatory events at the 'one determinant' level by using fragments of Fd that are devoid of one terminus (i.e., one antigenic determinant). Parallel studies will be done by using monospecific antibodies. Also, the nature and determinant specificity of immunoregulatory factors will be studied.

REFERENCES

1. Merryman, C. F., and P. H. Maurer. 1976. Genetic control of immune response against random copolymer of glutamic acid and alanine (GA) and tyrosine (GT) in inbred mice. J. Immunol. 116: 739.
2. Maurer, P. H., and C. F. Merryman. 1978. Immune responses of mice against poly (Glu, Lys, Ala) terpolymers and linkage with H-2. Immunogenetics 6:149.
3. Merryman, C. F., and P. H. Maurer. 1975. Characterization of a new Ir-GLT gene and its location in the I-region of the H-2 complex. Immunogenetics 1:549.
4. Merryman, C. F., P. H. Maurer, and J. H. Stimpfling. 1975. Unigenic and multigenic I region control of the immune response of mice to the GAT^{10} and GLϕ-GLT terpolymers. Immunogenetics 2:441.
5. Dorf, M. E., J. H. Stimpfling, and B. Benacerraf. 1975. Requirements for two H-2 complex Ir genes for the immune response to the L-Glu, L-Lys, L-Phe terpolymer. J. Exp. Med. 141:1459.
6. Schwartz, R. H., M. E. Dorf, B. Benacerraf, and W. E. Paul. 1976. The requirement for two complementing Ir-GLϕ immune response genes in the T-lymphocyte proliferative response to poly (Glu^{51}, Lys^{36}, Phe^{11}). J. Exp. Med. 143:897.
7. Deak, B. D., D. Meruelo, and H. O. McDevitt. 1978. Expression of a single histocompatibility complex locus controls the immune response to poly-L-(Tyrosine, Glutamic acid)-poly-D,L-Alanine-poly-L-lysine. J. Exp. Med. 147:599.
8. Merryman, C. F., P. H. Maurer, and A. R. Zeiger. 1977. Genetic control of two independently segregating loci in mice to the sequential polypeptide (Tyr-Ala-Glu-Gly). Immunogenetics 4:373.
9. Singh, B., E. Fraga, and M. A. Barton. 1978. Characterization and genetic control of the immune response to synthetic polypeptide antigens of defined geometry. J. Immunol. 121:784.
10. Schwartz, R. H., C. F. Merryman, and P. H. Maurer. 1979. Gene complementation in the T-lymphocyte proliferative response to poly-(Glu^{57} Lys^{38} Tyr^{5}): evidence for effects of polymer handling and gene dose. J. Immunol. 123:272.
11. Lozner, E. C., D. H. Sachs, and G. M. Shearer. 1974. Genetic control of the immune response to staphylococcal nuclease. I. Ir-Nase: control of the antibody response to nuclease by the Ir region of the mouse H-2 complex. J. Exp. Med. 139:1204.
12. Schwartz, R. H., J. A. Berzofsky, C. L. Horton, A. N. Schechter, and D. H. Sachs. 1978. Genetic control of the T lymphocyte proliferative response to staphylococcal nuclease: evidence for multiple MHC-linked Ir gene control. J. Immunol. 120:1741.
13. Keck, K. 1975. Ir gene control of immunogenicity of insulin and A-chain loop as a carrier determinant. Nature 254:78.
14. Rosenwasser, J. J., M. A. Barcinski, R. H. Schwartz, and A. S. Rosenthal. 1979. Immune response gene control of determinant selection. II. Genetic control of the murine T lymphocyte proliferative response to insulin. J. Immunol. 123:471.
15. Hill, S. W., and E. E. Sercarz. 1975. Fine specificity of the H-2 linked immune response gene for gallinaceous lysozymes. Eur. J. Immunol. 5:317.
16. Schwartz, R. H., A. M. Solinger, M. Ultee, and E. Margoliash. 1978. Genetic control of T-lymphocyte proliferative response to cytochrome C. Adv. Exp. Med. Biol. 98:371.
17. Melchers, I., and K. Rajewski. 1975. Specific control of responsiveness by two Ir loci in the H-2 complex. Eur. J. Immunol. 5:753.
18. Berzofsky, J. A. 1978. Genetic control of the immune response to mammalian myoglobins in mice. I. More than one I-region gene in H-2 controls the antibody response. J. Immunol. 120:360.
19. Okuda, U., P. R. Christadoss, S. Twining, M. Z. Atassi, and C. S. David. 1978. Genetic control of immune response to sperm whale myoglobin in mice. I. T lymphocyte proliferative response under H-2 linked Ir gene control. J. Immunol. 121:866.
20. Kelly, B., and J. G. Levy. 1978. Immunobiologic properties of the major antigenic determinants of the ferredoxin molecule. *In* Immunobiology of Proteins and Peptides. I. Edited by Stavitsky, M. Z., and A. B. Stavitsky. Plenum Publishing Corporation, New York. P. 181.
21. Lee, K. C., B. Singh, M. A. Barton, A. Procyshyn, and M. Wong. 1979. A simple reliable system for studying antigen-specific murine T-cell proliferation. J. Immunol. Methods. 25:159.
22. Voller, A., D. Bidwell, and A. Bartlett. 1970. Microplate enzyme immunoassays for the immunodiagnosis of viral infections. *In* Manual of Clinical Immunology. Edited by N. Rose, and H. Freedman. American Soc. Microbiol., Washington. P. 506.
23. Mortenson, L. E. 1964. Purification and analysis of ferredoxin from *Clostridium pasteurianum*. Biochim. Biophys. Acta 81:71.
24. Tanaka, M., T. Nakashima, H. F. Mower, and K. T. Yasunobu. 1963. The C and N-terminal amino acid sequence of *Clostridium pasteurianum* ferredoxin. Arch. Biochem. Biophys. 105:570.
25. Nitz, R. M., B. Mitchell, J. Gerwing, and J. Christensen. 1969. Studies on the antigenicity of bacterial ferredoxins. I. Effect of modification of cysteinyl residues on the antigenicity of *Clostridium pasteurianum* ferredoxin. J. Immunol. 103:319.
26. Kelly, B. S., J. G. Levy, and L. Sikora. 1979. The use of the enzyme-linked immunosorbent assay (ELISA) for the detection and quantification of specific antibody from cell cultures. Immunology 37:45.
27. Greenwood, F. G., W. M. Hunter, and J. J. Glover. 1963. The preparation of ^{131}I-labeled human growth hormone of high specific radioactivity. Biochem. J. 89:114.
28. Schwartz, R. H., and W. E. Paul. 1976. T-lymphocyte-enriched murine peritoneal exudate cells. II. Genetic control of antigen-induced T-lymphocyte proliferation. J. Exp. Med. 143:529.
29. 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 antibody response of mice to poly-L-(Tyr, Glu)-poly D,L-Ala-poly-L-Lys. J. Exp. Med. 135:126.
30. Cheung, N. K. V., M. E. Dorf, and B. Benacerraf. 1977. Genetic control of the primary humoral response to Glu^{56} Lys^{35} Phe^{9}. Immunogenetics 4:163.
31. Dorf, M. E., J. H. Stimpfling, and B. Benacerraf. 1979. Gene dose effect in Ir-gene-controlled systems. J. Immunol. 123:269.