Brief Definitive Report

INDUCTION OF A RAGWEED-SPECIFIC ALLERGIC STATE IN *Ir*-GENE-RESTRICTED NONRESPONDER MICE*

By NICHOLAS CHIORAZZI,‡ AMAR S. TUNG,§ AND DAVID H. KATZ

(From The Department of Cellular and Developmental Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037)

A series of recent experiments from our laboratory have focused on the demonstration and characterization of a relatively IgE class-specific, nonantigen-specific, cellular suppressor mechanism involved in the murine IgE antibody response to allergens not controlled by *H-2*-linked immune response (*Ir*) genes (1, 2). Such a suppressor T-cell mechanism was initially demonstrated indirectly by administering appropriate doses of ionizing X irradiation and cyclophosphamide at various stages in the IgE immune response (1). More direct evidence was derived from transfer experiments utilizing thymocytes or splenic theta-bearing cells to dampen the enhanced reaginic response induced by irradiation and cyclophosphamide (2). Furthermore, these suppressor cells exert their major effect by impeding T-helper cell induction although, as mentioned, sufficient numbers are capable of suppressing an ongoing IgE antibody response.

It appears that the number and/or potencies of such cells varies among murine inbred strains without relatinship to *H-2* haplotype since those animals which are "high" responders (e.g., BALB/c) of the IgE class to most allergens produce only moderately higher titers of specific reaginic antibody after elimination of this nonspecific suppressor mechanism, whereas "low" responders (e.g. AKR) or "nonresponders" (SJL) produce dramatically elevated titers of IgE antibody (1, 2); indeed, the latter mice produce levels which are equal to those of high responder mice which have not been manipulated. These latter observations demonstrated quite emphatically that under these experimental conditions "nonresponsiveness" of the IgE class (not related to responses to antigens controlled by *Ir* genes) is not the phenotypic expression of a genetic inability of certain strains to respond, but rather of their genetic capability to actively suppress reagin production.

On the basis of these collective observations, experiments were designed to ascertain whether a similar suppressor mechanism might be operative in those situations in which nonresponsiveness of the IgE class is not polyclonal but

* This is publication no. 1303 from the Immunology Departments and publication no. 25 from the Department of Cellular and Developmental Immunology, Scripps Clinic and Research Foundation, La Jolla, California. This work was supported by NIH grant AI-13874.

‡ Supported by NIH National Research Service Award no. 7-F32-AI 01965-02. Present address: Rockefeller University, New York 10021.

§ Supported by a Research Fellowship of the Cancer Research Institute (terminated November 1, 1976) and of the Arthritis Foundation (commenced November 1, 1976).

rather restricted to certain antigens which appear to be under histocompatibility linked genetic control. Indeed, the data presented herein demonstrate that nonantigen-specific suppressive phenomena are responsible, at least in part, for the lack of IgE responses to crude ragweed extract and its dinitrophenylated derivative by *Ir*-gene-restricted, nonresponder mice of the *H-2*^{b} haplotype.

Materials and Methods

Proteins and Hapten-Protein Conjugates. Ragweed pollen extract (RE; Greer Laboratories, Inc., Lenoir, N. C.) and crystallized bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, Mo.) were haptenated as previously described (3) and the following conjugates employed: $DNP_{4.5}$-RE and DNP_{26}-BSA.

Animals. 8- to 12-wk-old C57BL/6 and C57BL/10 mice of both sexes were obtained from The Jackson Laboratory, Bar Harbor, Maine. Male CD rats were purchased from Charles River Breeding Laboratories, Wilmington, Mass.

X Irradiation. Mice were exposed to total body X irradiation according to conditions previously described (4).

Administration of Cyclophosphamide (CY). CY (Mead Johnson & Co., Evansville, Ind.) was administered intraperitoneally at a dose of 100 mg/kg of body weight, 3 days before priming with RE.

Measurement of Anti-RE and Anti-DNP Antibodies

IgE ANTIBODIES. Serum IgE anti-RE and anti-DNP antibodies were assayed semiquantitatively by passive cutaneous anaphylaxis (PCA) reactions in rats by a modification (5) of the technique of Mota and Wong (6).

IgG ANTIBODIES. IgG anti-DNP antibodies in micrograms per milliliter were determined from sera of individual mice by a modified Farr technique (7) using ^{3}H-ε-amino-*N*-caproic acid (8).

Statistical Analysis. Reproducible differences of pools of IgE antibody of two or more two-fold dilutions (equal to or greater than 400% of the control response) were considered significant. Serum IgG anti-DNP levels in micrograms per milliliter were logarithmically transformed, and then geometric means and standard errors calculated. Group comparisons were made using the Student's *t* test.

For convenience of presentation and comparison, IgE levels have been displayed graphically as percentage of the control response. The actual PCA titer is listed on each graph adjacent to the appropriate control bar.

Results

Elicitation of IgE anti-Ragweed Antibody Response in H-2^b *Nonresponder Mice.* Previously, Dorf et al. (9) demonstrated that murine IgE and IgG antibody responses to RE and its 2,4-dinitrophenylated derivative (DNP-RE) involve a linkage to the major histocompatibility locus of the various inbred strains. Thus, mice of the *H-2*^{a,d,h,k,m,n,p} genotypes develop both primary and secondary IgE anti-RE antibody when immunized in the appropriate manner; on the contrary, mice of the *H-2*^{b,f,i,k,u} genotypes fail to produce anti-RE reaginic antibody under these same conditions.

In an attempt to test the hypothesis that suppressor cell activity may be responsible for this impaired synthesis of IgE antibody after exposure to RE, C57BL/6 nonresponder mice (*H-2* ^{b}) were either not manipulated (group I) or treated with 150 R of ionizing X irradiation on day 0 (group II) or 100 mg/kg of CY on day −3 (group III). These manipulations have been shown to result in enhanced IgE antibody formation in response to T-dependent, non-*Ir*-gene-restricted antigens in high, low, and nonresponder mice (1, 2). All of the experimental groups were primarily immunized with RE on day 0, secondarily challenged on day 7, and assayed for specific IgE anti-RE antibody 10 days later.

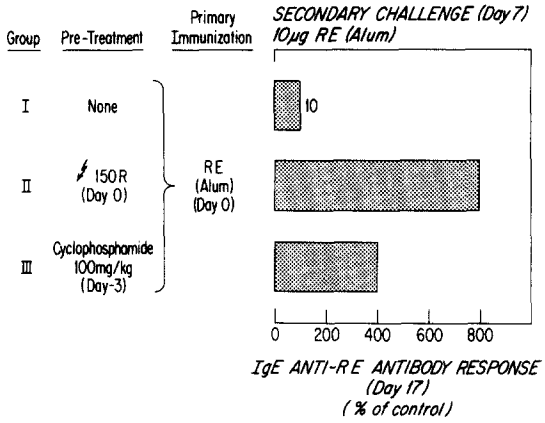

FIG. 1. Elicitation of IgE anti-ragweed antibody responses in C57BL/6 ($H\text{-}2^b$) nonresponder mice. Three groups of unprimed C57BL/6 mice (five animals per group) were either not treated or exposed to 150 R irradiation on day 0 or 100 mg/kg body weight of CY on day −3. All animals were primed with 10 μg of RE in 4 mg alum on day 7. The IgE anti-RE responses on day 17 are represented as percent of the control response with the actual PCA titer listed adjacent to the control bar.

Fig. 1 demonstrates that under these experimental conditions C57BL/6 mice produce negligible amounts of RE-specific reaginic antibody. These data are in accord with those of Dorf et al. (9). However, those animals manipulated in a manner to abrogate suppressor cell effects (e.g. irradiation, group II; and CY, group III) develop eight- and fourfold higher IgE anti-RE responses, respectively. A series of similar experiments utilizing both C57BL/6 and C57BL/10 mice reproducibly have yielded PCA titers 4- to 16-fold higher than control levels.

Demonstration of Helper T-Cell Activity for IgE anti-DNP Antibody Responses in RE Nonresponder Mice Immunized with DNP-RE. Since murine *Ir* genes appear to regulate antibody responses primarily at the T-cell level, the degree of helper T-cell function generated in this same *Ir*-gene-restricted nonresponder system before and after treatment with either X irradiation or CY was investigated. Thus, C57BL/6 mice were either not treated (group I) or treated on day 0 with X irradiation (group II) or on day −3 with CY (group III). All three groups were carrier primed with RE on day 0 and subsequently primed with DNP-RE on day 7. Fig. 2 illustrates the DNP-specific IgE antibody responses of these three groups 10 days after priming with DNP-RE.

Note the striking enhancement of IgE anti-DNP antibody produced in those animals treated with either X irradiation (8-fold) or CY (16-fold) as compared with the barely detectable responses of the untreated control mice. It is clear that pretreatment of these RE nonresponder mice has allowed quite significant helper cell priming and thereby good cooperative anti-DNP responses to DNP-RE. Again, similar data (not shown) were obtained in studies with C57BL/10 mice. There were no statistical differences in the IgG anti-DNP antibody levels of any of the groups (data not shown).

Discussion

These experiments clearly demonstrate that "*Ir*-gene-restricted" C57BL mice

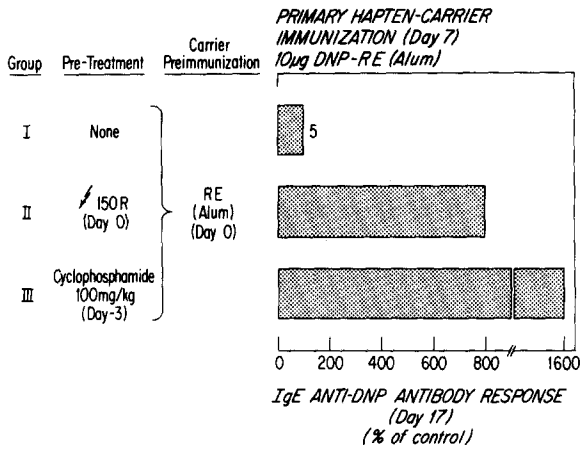

FIG. 2. Demonstration of T-helper activity for IgE anti-DNP responses in RE nonresponder mice challenged with DNP-RE. Protocol is identical to that in Fig. 1 except that all animals were boosted with 10 μg of DNP-RE on day 7. The IgE anti-DNP responses on day 17 are represented as percent of the control bar. IgG anti-DNP responses, though meager, were not statistically significant amongst the groups (data not shown).

can produce significant titers of ragweed-specific IgE antibody after treatment with doses of X irradiation or CY which presumably act by abrogating suppressor cell function (1, 2, 10–12). Furthermore, at least one point of action of these putative suppressor cells in the IgE response to RE and DNP-RE is at the level of helper cell induction, since elimination of these suppressive effects allowed the induction of and/or cooperative interaction between RE-primed T cells and unprimed DNP-specific B cells (Fig. 2). These observations are analogous to those reported earlier by us for IgE responses to DNP-ASC by SJL and AKR mice (2), strains which are genetically poor responders to all allergens without reference to their histocompatibility genotype (13), and suggest that a similar mechanism of nonantigen-specific T-cell-related suppression exists. However, it appears that such suppressor cells are not the sole factor determining the degree of reaginic response to RE and DNP-RE since the magnitude of enhancement of IgE antibody responses of C57BL/6 mice treated with X irradiation or CY to DNP-RE (4- to 8-fold) was generally less than that seen in responses to DNP-ASC (8- to 32-fold), an antigen not under genetic control (data not shown).

In view of these observations, it is clear that structural genes for IgE anti-RE antibody are indeed encoded in the genome of C57BL mice. Furthermore, it appears that their phenotypic expression is regulated by two mechanisms. The first mechanism is not related to *Ir*-gene phenomenology and is apparently operative at all times for all antigens. On the basis of our previous data (2) and that presented here, we suggest that nonantigen-specific suppressor T cells mediate this process. The second mechanism is the classical *Ir*-gene restriction and applies to selective antigens such as the one utilized in these experiments.

In the experiments reported here we have not seen comparable effects on nonreaginic, IgG responses to RE. However, Debré et al. (14) have reported that 10-fold higher IgG PFC responses can be elicited to the copolymer of glutamic acid and tyrosine (GT) in *Ir*-gene-restricted BALB/c mice after the administration of CY, although the number of PFC generated were approximately 50% of

those seen after pretreatment with CY and subsequent immunization with the immunogenic form of GT (e.g., GT-methylated BSA). Furthermore, T-cell-mediated responses appear to be similarly affected by this suppressor mechanism since Miller et al. (12) have shown that transient delayed-type hypersensitivity responses to LDH_B can be induced in nonresponder mice after administration of CY. In view of these collective observations, it is clear that previous concepts of *Ir*-gene-controlled unresponsiveness, which formally considered that nonresponsiveness implied a genetic deletion of dominant structural alleles, must be reinterpreted to take such findings into account.

Summary

Mice of the inbred strains, C57BL/6 and C57BL/10 ($H\text{-}2^b$), are genetically incapable of developing IgE antibody responses to ragweed pollen extract (RE) or its dinitrophenylated derivative, DNP-RE. This nonresponsiveness has previously been thought to reflect the absence of a relevant *H-2*-linked *Ir* genes controlling responses of inbred mice to these antigens. However, pretreatment of $H\text{-}2^b$ mice with either low doses of ionizing X irradiation or cyclophosphamide abrogates the nonresponder status of such animals, apparently by removal of a suppressive mechanism normally inhibiting development of IgE responses to these antigens. The implications of these findings for mechanisms of genetic control of IgE antibody synthesis and the *Ir*-gene concept are discussed.

We wish to thank Ms. Nomi Eshhar and Ms. Lee Katz for skilled technical assistance and Ms. Judy Henneke for excellent secretarial assistance in the preparation of the manuscript.

Received for publication 21 April 1977.

References

1. Chiorazzi, N., D. A. Fox, and D. H. Katz. 1976. Hapten-specific IgE antibody responses in mice. VI. Selective enhancement of IgE antibody production by low doses of X-irradiation and by cyclophosphamide. *J. Immunol.* 117:1629.
2. Chiorazzi, N., D. A. Fox, and D. H. Katz. 1977. Hapten-specific IgE antibody responses in mice. VII. Conversion of IgE "non-responder" strains to IgE "responders" by elimination of suppressor T cell activity. *J. Immunol.* 118:48.
3. Hamaoka, T., D. H. Katz, and B. Benacerraf. 1973. Hapten-specific IgE antibody responses in mice. II. Cooperative interactions between adoptively transferred T and B lymphocytes in the development of IgE responses. *J. Exp. Med.* 138:538.
4. Fox, D. A., N. Chiorazzi, and D. H. Katz. 1976. Hapten-specific IgE antibody responses in mice. V. Differential resistance of IgE and IgG B lymphocytes to X-irradiation. *J. Immunol.* 117:1622.
5. Hamaoka, T., P. E. Newburger, D. H. Katz, and B. Benacerraf. 1974. Hapten-specific IgE antibody responses in mice. III. Establishment of parameters for generation of helper T cell function regulating the primary and secondary responses of IgE and IgG B lymphocytes. *J. Immunol.* 113:958.
6. Mota, I., and D. Wong. 1969. Homologous and heterologous passive cutaneous anaphylactic activity of mouse antisera during the course of immunization. *Life Sci.* 8:813.
7. Farr, R. S. 1958. A quantitative immunochemical measure of the primary interaction between I*BSA and antibody. *J. Infect. Dis.* 108:329.
8. Katz, D. H., W. E. Paul, E. A. Goidl, and B. Benacerraf. 1970. Carrier function in

anti-hapten immune responses. I. Enhancement of primary and secondary anti-hapten antibody responses by carrier preimmunization. *J. Exp. Med.* 132:261.

9. Dorf, M. E., P. E. Newburger, T. Hamaoka, D. H. Katz, and B. Benacerraf. 1974. Characterization of an immune response gene in mice controlling IgE and IgG antibody responses to ragweed pollen extract and its DNP derivative. *Eur. J. Immunol.* 4:346.
10. Polak, L., and J. L. Turk. 1974. Reversal of immunological tolerance by cyclophosphamide through inhibition of suppressor cell activity. *Nature (Lond.).* 249:654.
11. Askenase, P. W., B. J. Hayden, and R. K. Gershon. 1975. Augmentation of delayed-type hypersensitivity by doses of cyclophosphamide which do not affect antibody responses. *J. Exp. Med.* 141:697.
12. Miller, J. F. A. P., M. A. Vadas, A. Whitelaw, and J. Gamble. 1976. *H-2* linked *Ir* gene regulation of delayed-type hypersensitivity in mice. *In* The Role of Products of the Histocompatibility Gene Complex in Immune Responses. D. H. Katz and B. Benacerraf, editors. Academic Press, Inc., New York. 579.
13. Levine, B. B., and N. M. Vaz. 1970. Effect of combinations of inbred strain, antigen, and antigen dose on immune responsiveness and reagin production in the mouse. *Int. Arch. Allergy Appl. Immunol.* 30:156.
14. Debré, P. D., C. Waltenbaugh, M. E. Dorf, and B. Benacerraf. 1976. Genetic control of specific immune suppression. IV. Responsiveness to the random copolymer L-glutamic acid^{50}-L-tyrosine^{50} induced in BALB/c mice by cyclophosphamide. *J. Exp. Med.* 144:277.