# HAPTEN-SPECIFIC IgE ANTIBODY RESPONSES IN MICE

## I. Secondary IgE Responses in Irradiated Recipients of Syngeneic Primed Spleen Cells*

By TOSHIYUKI HAMAOKA, DAVID H. KATZ, KURT J. BLOCH, and BARUJ BENACERRAF

(*From the Department of Pathology and Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115*)

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The induction of humoral immune responses to a variety of antigens requires the cooperative participation of both thymus-derived (T) and bone marrow-derived (B) lymphocytes. The former cells exert very definite regulatory influences on the responses of IgM and IgG B lymphocyte precursors of antibody-forming cells with respect to triggering and differentiative events after exposure to antigen (reviewed in ref. 1). An understanding of T cell regulation of IgE antibody responses, in particular, would be highly desirable because of its practical application for the control of many allergic diseases.

Evidence obtained in recent studies by other investigators (2–6) has strongly indicated the participation of T cell functions in hapten-specific IgE antibody responses in rats and rabbits. Studies of IgE antibody production in mice have demonstrated a histocompatibility-linked genetic control over responses of this immunoglobulin class to certain antigens (7) which, by analogy with other well-defined genetically-controlled specific immune responses (8), also implies a necessary role for T cell participation. However, the nature of T cell regulation of IgE antibody production, if it indeed exists, is largely unknown. Specifically, a crucial question concerns whether or not the effect of activated helper T cells on IgE production follows the same general pattern, both qualitatively and quantitatively observed in the elicitation of IgG antibody responses and, by extension, whether the same or different T cells operate on the two antibody class precursor lymphocytes, respectively.

In the present report, we describe the successful transfer to irradiated recipients, in several strains of inbred mice, of specific IgE antibody responses to the hapten 2,4-dinitrophenyl (DNP) conjugated to different protein carriers, using spleen cells from syngeneic donors sensitized to produce IgE antibodies. This system will permit further detailed analysis of the activities of specific B and T lymphocytes, respectively, in the generation of IgE as compared to IgG antibody responses.

### *Materials and Methods*

*Proteins and Hapten-Protein Conjugates.*—Keyhole limpet hemocyanin (KLH) was purchased from Pacific Bio-Marine Supply Co., Venice, Calif. Ascaris proteins (ASC) were ex-

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tracted from *Ascaris suum* as described by others (9). Bovine gamma globulin (BGG) was obtained from Pentex Biochemical, Kankakee, Ill. The following DNP conjugates were prepared as previously described (10, 11): $DNP_{14}$-KLH, $DNP_{2.1}$-ASC, and $DNP_{32}$-BGG. Subscripts refer to the number of moles of DNP per 100,000 molecular weight units of KLH; per mole of carrier for BGG and moles of DNP $\times 10^{-7}$ per milligram of ASC protein.

*Animals.*—Mice of the inbred lines A/J, BALB/c, and (A $\times$ BALB/c)$F_1$ hybrids ($CAF_1$) were obtained from the Jackson Laboratories, Bar Harbor, Maine. All mice were immunized at 8–12 wk of age. Random-bred white female CFW mice used to assay IgE antibody titers were obtained from Carworth Farms, New City, N. Y.

*Immunizations.*—Mice were primarily immunized by intraperitoneal injection of either DNP-KLH (2 $\mu$g) or DNP-ASC (1 or 10 $\mu$g) mixed with 2–10 mg of $Al(OH)_3$ gel in a total volume of 0.5 ml of saline. $Al(OH)_3$ gel (alum) was prepared by mixing equal volumes of 2N $Al_2(SO_4)_3$ and 2N NaOH as described by Levine and Vaz (7). Mixtures of hapten-protein conjugates with alum were prepared immediately before use. In some experiments the mice were secondarily immunized using the same dose and route of administration of antigen-alum mixture. Mice were bled from the retro-orbital venous plexus at appropriate intervals after primary and/or secondary challenge and serum levels of IgE and IgG anti-DNP antibodies were determined as described below.

*Adoptive Cell Transfer System.*—Mice were primed with either 1–2 $\mu$g of DNP-KLH or 10 $\mu$g of DNP-ASC mixed with 2 or 10 mg of alum. In some instances booster injections were administered 4 wk after primary immunization. 4 wk after the primary or secondary immunization, these DNP-primed mice were killed and their spleens removed. Single cell suspensions in Eagle's minimum essential medium (MEM) were prepared, washed and transferred intravenously to syngeneic, irradiated (500 r) recipients (50 $\times$ $10^6$ cells per recipient). Immediately after cell transfer, secondary challenge was performed intraperitoneally with a DNP-protein conjugate mixed with alum. All mice were bled 7 days after cell transfer and challenge and serum anti-DNP antibody levels were determined.

*Measurement of Anti-DNP Antibodies.*—

*IgE antibodies:* The level of reaginic (IgE) anti-DNP antibodies in pools of sera from groups of five mice were determined by passive cutaneous anaphylaxis (PCA) reactions using shaven CFW test mice as described by Levine and Vaz (7). Briefly, 25 $\mu$l aliquots of serum from each mouse within a given group were pooled and serially diluted (twofold) in saline. 50 $\mu$l of each serum dilution were injected intradermally into the dorsal skin surface of the test mice. 48 h after intradermal sensitization, DNP-specific PCA reactions were elicited by intravenous injection of 500 $\mu$g of DNP-BGG in 0.3 ml of 0.5% Evans blue dye dissolved in 5% dextrose-water solution. Reactions were read and recorded as the reciprocal of the highest dilution of serum evoking threshold PCA reactivity (5 mm diameter). Sera which failed to elicit detectable PCA reactions were arbitrarily assigned a value of $<5$.

Two points of importance should be emphasized about the IgE assay. Firstly, the PCA sensitizing antibody being measured here is labile to heating at 56°C for 2 h, and is optimally detected 48 h after intradermal sensitization of the test mice. These are characteristic properties of reaginic antibodies of the IgE class and rule out any contribution of skin-sensitizing antibodies of the IgG1 antibody class (12). Secondly, the DNP-specificity of the IgE antibodies is verified by the fact that a DNP-conjugate of an unrelated carrier protein (BGG) has been used to test for such antibodies (under condition where the BGG carrier molecule alone fails to elicit a positive PCA), and moreover, that the PCA reactions could be specifically inhibited with univalent DNP-L-lysine.

*IgG serum antibodies:* Serum IgG anti-DNP antibody levels were determined in individual sera by a modified Farr technique (13) using [$^3$H]DNP-$\epsilon$-amino-*N*-caproic acid as previously described (10).

## RESULTS AND DISCUSSION

*Elicitation of DNP-Specific IgE Antibody Responses in Mice.*—Groups of A/J, BALB/c, and $CAF_1$ mice were primarily immunized with either 1 or 10

μg of DNP-ASC plus alum (10 mg) or 2 μg of DNP-KLH plus alum (2 mg). All animals received a second injection of antigen-alum mixture (same dose) 30 days after primary immunization. Mice were bled at 10, 20, and 30 days after primary immunization, and again 7 days after the secondary challenge. The levels of IgE antibody production in the three strains of mice employed are depicted graphically in Fig. 1.

All strains tested made easily detectable DNP-specific IgE antibody responses by day 10 after primary immunization with small doses of either DNP-ASC or DNP-KLH. Although the magnitude of IgE antibody production differed among the strains and with respect to the antigen and dose employed, the kinetic patterns of response were strikingly similar. Thus, peak antibody production was attained around 20 days after primary immunization, after

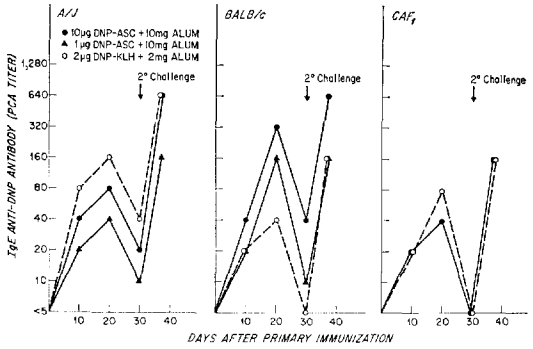


FIG. 1. Elicitation of DNP-specific IgE antibody responses in mice. Groups of five mice of the inbred lines A/J, BALB/c, and $CAF_1$ hybrids were immunized intraperitoneally with either DNP-ASC or DNP-KLH plus alum in the doses indicated. The mice were bled on days 10, 20, and 30 at which time secondary challenge with the same antigen and dose was performed. A final bleeding was made 7 days after secondary challenge. Levels of serum IgE anti-DNP antibody at the various times of bleeding are illustrated.

which the IgE titers in serum diminished. Although not shown in Fig. 1, other experiments in our laboratory on kinetics of the primary response have demonstrated that serum IgE titers diminish to undetectable levels by day 40 or 50 after a single immunization in all three of these mouse strains. On the other hand, administration of a secondary challenge on day 30 elicited very good anamnestic IgE anti-DNP antibody responses in all strains (Fig. 1), provided such challenge was made with the original immunizing DNP-conjugate. Challenge with an unrelated DNP-carrier conjugate consistently failed to induce a secondary IgE anti-DNP response.

*Adoptive Transfer of DNP-Specific IgE Antibody Responses With DNP-Primed Spleen Cells.*—The capacity to elicit very brisk anamnestic IgE anti-DNP antibody responses with the homologous DNP-protein used for priming provides a clear indication of the development of a specific IgE memory cell pool under these conditions of priming. This essential requisite for adoptive immunity experiments having been demonstrated, we were then able to design

and test experimental conditions capable of allowing successful adoptive transfer of the IgE anti-DNP response.

Several experiments of this type were performed. The protocol and results of three such experiments are summarized in Table I. In the first, spleen cells from A/J donor mice that had been primed with DNP-ASC (10 $\mu$g) plus 10 mg alum were injected intravenously into syngeneic, irradiated recipients. Two types of primed spleen cells were used as indicated in the table. A control group of recipients were injected with spleen cells from nonimmunized syngeneic donors. Secondary challenge with 10 $\mu$g of DNP-ASC plus 1 mg alum was performed immediately after cell transfer. Curiously, the best adoptive secondary IgE responses to DNP-ASC were displayed by recipients of cells from singularly primed donors; nonetheless, clear adoptive secondary responses were elicited in recipients of cells from primed and boosted donors. As expected, no IgE antibodies were detected in control mice that had either received normal spleen cells or had not been secondarily challenged.

A similar experiment employing the same priming regimen was carried out in BALB/c mice. Once again, very good adoptive secondary IgE anti-DNP antibody responses were elicited in recipients of primed cells which were challenged with DNP-ASC whereas control mice either not challenged or injected with normal rather than primed donor cells failed to manifest detectable IgE serum antibodies. Comparable results were obtained when A/J donors were primed with a single dose of 1 $\mu$g DNP-KLH plus 10 mg alum 8 wk before cell transfer. Secondary challenge of recipients with DNP-KLH plus alum elicited very good DNP-specific IgE responses, whereas again appropriate control groups made no detectable IgE antibody.

The IgG antibody responses obtained in all of the adoptive transfer experiments were quite high in magnitude after challenge with the homologous antigen.

TABLE I

*Adoptive Transfer of DNP-Specific IgE Antibody Responses with DNP-Primed Spleen Cells*

| Mouse strain | Protocol | | | Serum anti-DNP antibody | |
|---|---|---|---|---|---|
| | Spleen cells transferred | Immunization | Secondary challenge | IgE (PCA)* | IgG‡ |
| | | | | | ($\mu$g/ml) |
| A/J | DNP-ASC-primed | 4 wk earlier | DNP-ASC | 2,560 | 1,068.5 |
| | DNP-ASC-primed | 4 wk earlier | None | <5 | 0.1 |
| | DNP-ASC-primed | 8 wk and 4 wk earlier | DNP-ASC | 320 | 5,521.8 |
| | DNP-ASC-primed | 8 wk and 4 wk earlier | None | <5 | 1.7 |
| | Normal | None | DNP-ASC | <5 | 0.1 |
| BALB/c | DNP-ASC-primed | 8 wk and 4 wk earlier | DNP-ASC | 2,560 | 3,741.9 |
| | DNP-ASC-primed | 8 wk and 4 wk earlier | None | <5 | 0.7 |
| | Normal | None | DNP-ASC | <5 | 0.1 |
| A/J | DNP-KLH-primed | 8 wk earlier | DNP-KLH | 5,120 | 642.8 |
| | DNP-KLH-primed | 8 wk earlier | None | <5 | 0.1 |
| | Normal | None | DNP-KLH | <5 | 0.1 |

* The data are expressed as the reciprocal of the highest PCA titer elicited by pooled sera from five mice of each recipient group bled 7 days after secondary challenge.

‡ The data are expressed as geometric mean anti-DNP antibody levels of sera from individual mice in each group of five recipients bled 7 days after secondary challenge.

This is particularly noteworthy in view of the fact that cell donors were primed without the use of bacterial adjuvants. The obviously high degree of priming obtained clearly reflects the immunogenic strength of the protein carriers, i.e. ASC and KLH, employed. It was also of interest that a clear dissociation occurred between magnitude of the IgE and IgG antibody responses, respectively, in the A/J recipients of spleen cells from donors that had been primed and boosted with DNP-ASC. Thus, whereas the IgG anti-DNP response was considerably higher than that obtained in recipients of singularly-primed donor cells, the IgE antibody response was conversely much lower.

The present studies have demonstrated the successful adoptive transfer of hapten-specific secondary responses of the IgE antibody class. Several points are noteworthy about this system: One less important point, but nevertheless somewhat surprising, was the relative ease with which adoptive secondary IgE responses could be obtained with transferred spleen cells. The successful transfer of IgE responses with primed spleen cells not only makes this system somewhat more practical for further studies, but also demonstrates clearly that all of the relevant participatory cells reside in the same organ environment known to contain the cells essential for development of responses of the IgG antibody class.

A second point concerns the magnitude of the IgE responses obtained after adoptive cell transfer. The PCA titers, in most instances, were considerably higher in the irradiated recipients of primed cells after secondary challenge than those observed in secondary responses of intact animals. There are probably two factors contributing to this observation: (*a*) the nonspecific proliferative stimulus existing in the environment of an irradiated recipient; and (*b*) the absence of the predominant target tissue cell, i.e., the mast cell, to which IgE molecules would normally attach. This latter explanation derives from the fact that mast cells are relatively radiation-sensitive (14) and, therefore, would be deficient in an irradiated recipient.

Finally, the adoptive transfer IgE response is remarkably similar to the adoptive transfer IgG antibody system in several respects. Thus, the optimal source of lymphocytes, the kinetics of anamnestic antibody production and, in general, the mode of donor priming are essentially identical for both types of antibody class responses. One exception to the latter was the dissociation of IgE and IgG adoptive secondary responses obtained with DNP-ASC-primed cells from primed and boosted A/J donor mice. This did not occur, however, with similarly primed cells in BALB/c mice. Most importantly, the requirement for carrier-specificity in the elicitation of anti-DNP secondary IgE responses is rigidly maintained. Thus, by analogy with in vivo IgG antibody responses in hapten-carrier systems, the participation of carrier-specific helper lymphocytes is clearly implied. This notion is consistent with the observations of Tada and Okumura in the rat (3), and of Kishimoto and Ishizaka in studies of in vitro IgE antibody production by rabbit lymph node lymphocytes (5). Further studies to delineate the nature of carrier-specific lymphocytes and the comparative features of their capacity to regulate responses of DNP-specific B

lymphocytes of the IgE and IgG antibody class, respectively, are currently underway.

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