

Long-Lived IgE- and IgG-Secreting Cells in Rodents Manifesting Persistent Antibody Responses

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Received April 5, 1984; accepted June 19, 1984

BALB/c mice and BN rats manifesting persistent IgE and IgG responses were examined up to 1 year after immunization. A significant proportion of the ongoing antibody response in these animals survived lethal X-irradiation employing dosages sufficient to deplete B memory cells. The persistent IgE responses in both species were refractory to exogenous isotype-specific suppressor cells taken from tolerant syngeneic animals, which were shown to abrogate primary IgE responses in parallel tests. Employing a novel ELISA-based assay for plaque forming cells, long-lived radioresistant IgE- and IgG-secreting cells were identified in differing ratios in lymph nodes, spleen, and bone marrow of both species. These long-lived cells were shown to arise following maximum antigenic challenge with antigen plus adjuvant, and after repeated low-grade stimulation by antigen alone, including passive inhalation of dilute antigen aerosols.

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1. INTRODUCTION

Persistent IgE antibody production in the apparent absence of constant antigenic stimulation, typified by the year-long high serum titers of anti-pollen antibody in many hay fever patients, remains a hallmark of human atopy (1, 2). Recent studies in mice have shown that comparable persistent IgE responses may be induced in some inbred strains, by immunization with minute doses of antigen plus an appropriate adjuvant (3). The bulk of IgE production in these mice continues after the animals have been subjected to 1000-R irradiation (4, 5), implying a role for long-lived plasma cells in the maintenance of persistent IgE responses in high-responder mice, and by analogy in atopic humans.

The detailed study of these potentially important cells has been hampered by the lack of a reliable assay system for the detection of IgE secretion. A recent report from this laboratory (6) detailed an ELISA-plaque-forming cell (PFC)² assay capable of visualizing the Ig fingerprint of individual antigen-specific plasma cells formed

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² Abbreviations used: PFC, plaque-forming cells; OA, ovalbumin; AH, aluminium hydroxide; DNP, 2,4-dinitrophenyl-; PBS, phosphate-buffered saline; PCA, passive cutaneous anaphylaxis; HA, hemagglutinating antibody; ELISA, enzyme-linked immunosorbent assay; 5-BCIP, 5-bromo-4-chloro-3-indolyl-phosphate; AP, Type VII alkaline phosphatase.

by incubating actively secreting PFC over a solid phase containing conjugated antigen. The assay has proven capable of detecting IgE-PFC in the lymph nodes of rats during primary IgE responses (7), and in the present study has been employed to map the distribution of long-lived radio-resistant IgE- and IgG-secreting cells in the organs of rats and mice displaying persistent antibody responses of up to 1 year's duration. Additionally, we confirm the reported insensitivity of these cells in the mouse to the modulatory effects of suppressor T cells (8), and extend these observations to high-IgE-responder rats.

2. MATERIALS AND METHODS

2.1 *Materials*

Ovalbumin (OA; Grade V) was obtained from Sigma Chemical Company (St. Louis, Mo.). Affinity-purified anti-immunoglobulin reagents for use with the ELISA-PFC assay employing rat cells were as detailed in (7); the affinity-purified rabbit anti-mouse IgE was a gift from Professor K. J. Turner. Type I agarose and Type VII-S alkaline phosphatase (AP) and the AP substrate 5-bromo-4-chloro-3-indolyl phosphate (5-BCIP) were all obtained from Sigma. Aluminium hydroxide (AH) was from Wyeth Pharmaceuticals (Australia).

2.2 *Animals and Treatments*

Male Brown Norway (BN) rats and female BALB/c mice were used. Intraperitoneal (ip) immunization of rats employed 100 μ g OA in PBS in conjunction with 10 mg AH (AH-OA); mice received either 10 μ g OA in PBS, 10 μ g OA together with 4.0 mg AH, or 10 μ g DNP-OA (conjugation ratio 3.4:1, prepared as in (9)) with AH. One group of rats was exposed once weekly for 7 min to an aerosol of 1% OA in PBS, as detailed elsewhere (10).

In X-irradiation experiments, rats received 850 R and mice 1000 R; the animals were given 5.0 and 2.0×10^7 syngeneic bone marrow cells, respectively, on the day of irradiation to ensure survival. X-irradiation was performed in a Siemens Stabilopan Kilovoltage X-ray machine under the following physical conditions: for rats, 270 kV, 12 mA, TH 1 Cu/Sn/Al filter, focal distance 50 cm, dose rate 55.8 R/min; for mice, 200 kV, 18 mA, 0.5-mm Cu filter, focal distance 50 cm, dose rate 66.7 R/min.

2.3 *Serum Antibody Assays*

IgE-anti-OA titers were determined by the passive cutaneous anaphylaxis (PCA) assay of Ovary (11), as detailed in (12). Hemagglutinating antibody (HA) titers were determined employing OA-conjugated sheep erythrocytes (12); HA in these experiments was predominantly IgG, on the basis of mercaptoethanol resistance. Statistical analysis of data was performed via Student's *t* test.

2.4 *ELISA-PFC Assay for IgG- and IgE-Secreting Cells*

The assay was as outlined in (6, 7) except that polymerized (poly) OA was used as a solid phase, rather than native OA. Polymerization was affected by the dropwise

addition of 2.5% glutaraldehyde (w/v) to OA dissolved in PBS at pH 7.5 to a final molar ratio of 25:1; during the ensuing 4 hr the pH was readjusted to 7.5 three times, and the reaction was terminated by the addition of a 50:1 molar excess of solid glycine. The product was clarified and lyophilized.

The assay for rat IgG and IgE via ELISA-PFC was as described (6, 7) and involved the incubation of mononuclear cell suspensions from spleens, lymph nodes, or bone marrow at 37 or 32°C in 24-well tissue culture plates, previously coated with poly OA (2.0 mg/ml) in bicarbonate coating buffer (13). After incubation, the plates were washed, and sequentially exposed to affinity-purified rabbit anti-rat IgE or semipurified anti-IgG, an AP conjugate of sheep anti-rabbit IgG, and finally the substrate 5-BCIP dissolved in 36°C-gelling agarose. Areas previously occupied by IgE- or IgG-secreting cells developed as macroscopic blue spots or plaques (Fig. 1). The procedure for mouse cells was comparable, except that 3–4 hr was found to be the optimal incubation period for development of both IgE and IgG ELISA plaques in this species. Additionally, some experiments enumerated IgE-anti-DNP-secreting cells; here, wells were precoated employing 2.0 mg/ml solutions of DNP-human serum albumin (13:1).

Details of the specificity of the anti-Ig reagents for rats are shown in (7). Comparable procedures were adopted for mice; additionally, the inclusion of purified mouse myeloma IgE-anti-DNP (14) at 100 µg/ml in the incubation steps employing anti-mouse IgE was found to abrogate development of IgE-anti-OA plaques while not affecting IgG plaques; conversely, purified mouse IgG blocked IgG plaques but not those formed by IgE-secreting cells.

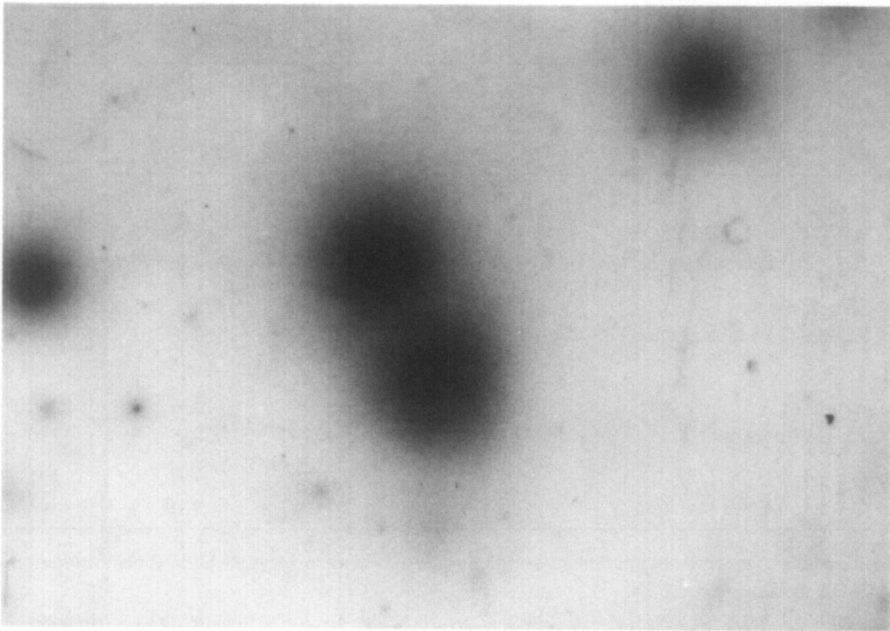


FIG. 1. ELISA plaques formed by plasma cells secreting IgE-anti-OA. The IgE plaques illustrated were developed following incubation of spleen cells from rats immunized with AH-OA in microplate wells containing conjugated OA, as detailed under Materials and Methods. Magnification $\times 100$.

2.5 Cell Preparation

Rat cells were prepared from spleens, lymph nodes, or bone marrow by dispersing tissues in chilled Dulbecco's PBS containing 5% fetal calf serum (FCS). Mouse cells were prepared from similar tissues in the buffered balanced salt solution of Von Boehmer and Shortman (15).

3. RESULTS

3.1 Radioresistance of Persistent Antibody Responses

High IgE-response BALB/c mice respond vigorously to a single ip injection of AH-OA, and 30 days later manifest high serum titers of anti-OA IgE and IgG antibodies which may persist for up to 1 year (see below). Lethal X-irradiation of these animals effected only partial reduction of these responses (Table 1a). Repeated ip challenge with soluble OA in the absence of adjuvant stimulated similar IgG and IgE responses, a large proportion of which were again radioresistant (Table 1b).

High IgE-response BN rats develop persistent IgE-anti-OA titers equivalent to 80–320 PCA units by 1 month following a single ip challenge with AH-OA, together with IgG responses comparable to those seen in mice. These responses are also

TABLE I
Radioresistant IgE and IgG Responses in Rats and Mice

Animals & treatments	Anti-OA titer (means $\pm$ SE; $n = 5$)			
	Day 0		Day + 35 ^a	
	PCA	HA	PCA	HA
BALB/c mice				
(a) AH-OA ^b	1536 $\pm$ 260	1280 $\pm$ 356	960 $\pm$ 168	720 $\pm$ 201
AH-OA plus radiation ^c	1536 $\pm$ 260	1280 $\pm$ 356	384 $\pm$ 72	448 $\pm$ 80
(b) Soluble OA ^d	all 1280	6656 $\pm$ 1560	1920 $\pm$ 905	3840 $\pm$ 823
Soluble OA plus radiation	all 1280	6656 $\pm$ 1560	960 $\pm$ 301	1600 $\pm$ 905
BN rats				
(c) AH-OA	80–320 ^f	3328 $\pm$ 1774	110 $\pm$ 31	2352 $\pm$ 830
AH-OA plus radiation ^c	80–320 ^f	3328 $\pm$ 1774	50 $\pm$ 9	1120 $\pm$ 145
(d) OA aerosol ^e	<10	819 $\pm$ 130	<10	921 $\pm$ 104
OA aerosol plus radiation	<10	1433 $\pm$ 255	<10	1843 $\pm$ 208

^a Comparable data were obtained from samples collected between Days 21 and 50 postirradiation.

^b AH-OA ip, Day –30.

^c X-Irradiated mice received 1000 R plus 2.0×10^7 syngeneic bone marrow cells. X-Irradiated rats received 850 R plus 5.0×10^7 syngeneic bone marrow cells.

^d Four monthly ip doses of 10 μ g soluble OA. The last dose was given 30 days before X-irradiation.

^e Weekly exposure.

^f Figures were not available for this experiment. Values shown are a range observed in other experiments.

stable in magnitude for many months (data not shown), and contain a significant component of irradiation-resistant antibody production (Table 1c). Repeated exposure to OA in the absence of adjuvant, in this case via repeated inhalation of an OA aerosol, triggered persistent IgG production (see 10), which appeared totally radio-resistant (Table 1d).

3.2 Failure of Suppressor Cells to Regulate Persistent IgE Responses

Previous work from this laboratory has shown that BALB/c mice repeatedly exposed to low levels of soluble OA by aerosol inhalation or by oronasal inoculation, develop antigen-specific tolerance to subsequent systemic challenge, which is restricted to the IgE isotype (16). Splenocytes from these animals contain isotype-specific suppressor cells which are sensitive to monoclonal anti-Thy 1.2 antiserum, and which abrogate adoptively transferred (17) or primary anti-OA responses (8). Figure 2B demonstrates that while active in animals undergoing primary immunization with AH-OA (group K < J; $P < 0.0001$), they are ineffective against persistent IgE-secreting cells (group G not significantly different from H and I).

Comparable data were obtained in parallel experiments with BN rats (Fig. 2A). In the latter, rats immunized 45 days previously with AH-OA (group C) displayed significant serum PCA and HA-anti-OA titers (viz, persistent IgE and IgG responses). These animals were given iv inoculations of 10^8 respiratory tract lymph node cells

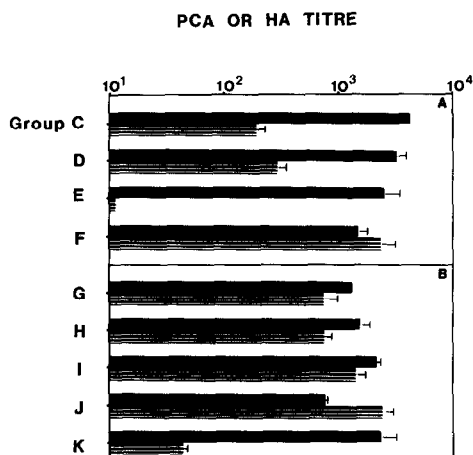


FIG. 2. Failure of IgE isotype-specific suppressor cells to inhibit persistent IgE responses. Data shown are means $\pm$ SD of reciprocal PCA (stripes) or HA (bars) titers, from groups ($n \geq 5$) of BN rats (A) or BALB/c mice (B). Group C received AH-OA ip on Day -45 and was bled on Day 0; group D (GpC rats) received 10^8 suppressor cells iv on Day 0 and was bled on Day 30; group E (normal rats) received 10^8 suppressor cells iv and AH-OA ip on Day 0 and was bled on Day 30; group F (normal rats) received AH-OA ip on Day 0 and was bled on Day 30. Groups G-I received AH-OA ip on Day -30; group G was bled Day 0; group H was bled on Day 30; group I was also given 5.0×10^7 suppressor cells iv on Day 0 and was bled on Day 30. Groups J and K (normal mice) were given AH-OA ip on Day 0; group J was bled on Day 30; group K was also give 5.0×10^7 suppressor cells iv on Day 0 and was bled on Day 30.

containing IgE isotype-specific and OA antigen-specific suppressor cells (prepared from syngeneic rats as in (10)). Serum PCA titers in these animals (group D) did not decline over the ensuing 30 days, whereas the same cellular inoculum given to naive controls prior to primary immunization with AH-OA selectively inhibited the IgE component of the ensuing primary anti-OA response (group E < F; $P < 0.001$).

3.3 Organ Location of Persistent IgE- and IgG-Secreting Cells

In BN rats immunized once with AH-OA ip and examined 60 days later, persistent serum titers of IgE and IgG-anti-OA were accompanied by varying numbers of antibody-secreting cells in different organs (Table 2). The major lymphatic drainage of the rat peritoneal cavity is to the parathymic lymph nodes (18), and we have recently shown that the primary IgE response in this species to

TABLE 2
Distribution of "Persistent" IgE- and IgG-Secreting Cells in Organs of Rats and Mice

Animals & Treatment	Serum titers (Day 0)		IgG- (IgE-)secreting cells/ 10^7 Cells (Day 0) ^a			
	PCA	HA	Bone marrow	Spleen	Mesenteric lymph nodes	Parathymic lymph nodes
Anti-OA						
BN rats						
(a) AH-OA (ip, Day -50); ($n = 5$)	80	2560	130 (0)	40 (2)	—	230 (43)
AH-OA plus 850 R (Day -21)	40	1280	80 (0)	90 (3)	—	220 (15)
(b) AH-OA (ip, Day -60)						
Animal 1	80	2560	1180 (7)	380 (0)	50 (0)	370 (220) ^b
Animal 2	320	5120	680 (0)	170 (10)	10 (0)	370 (220) ^b
Animal 3	320	5120	830 (0)	230 (3)	60 (0)	370 (220) ^b
BALB/c mice						
(c) AH-OA (ip, 1 year earlier; $n = 3$)	1280	320	80 (10)	20 (8)	20 (0)	—
(d) Soluble OA ($n = 5$) ^c	1280	2560	1620 (36)	430 (19)	40 (0)	—
Soluble OA plus 1000 R (Day -50)	1280	5120	630 (23)	150 (26)	20 (6)	—
Anti-DNP						
(e) AH-DNP/OA (ip, Day - 92)						
Animal 1	1280	—	680 (17)	660 (7)	60 (3)	—
Animal 2	1280	—	510 (7)	320 (16)	0 (3)	—
Animal 3	1280	—	340 (16)	260 (0)	0 (0)	—

^a Via ELISA-PFC assay.

^b Pool from the three animals.

^c Four ip doses of 10 μ g soluble OA.

ip challenge with AH-OA arises in these nodes (7). Persistent IgE-secreting cells appear to be largely restricted to these same nodes in normal (Table 2b) or X-irradiated (Table 2a) animals. In marked contrast, the bone marrow and to a lesser extent the spleen appear to be major sites of persistent IgG plasma cells in this species.

In BALB/c mice immunized under similar conditions with either OA or the hapten DNP, the bone marrow and spleen appear as the principal sites for both long-lived IgE- and IgG-secreting cells (Table 2c and 2e). Similar results were obtained employing cell preparations from mice repeatedly challenged with soluble OA without adjuvant (Table 2d). The small numbers of cells recovered from BALB/c parathymic lymph nodes argued against their examination in these assays.

4. DISCUSSION

Persistent antibody formation against other than replicating antigens is generally considered to result from the constant recruitment of B memory cells into the response via stable pools of antigen trapped in dendritic-like cells in lymph nodes (19–23). This view is not, however, consistent with an equally wide body of evidence (reviewed in (24)) on the radioresistance of established antibody responses. In the context of IgE, this thesis is directly challenged by the findings from Ishizaka's laboratory (4, 25) and subsequently from other laboratories (5, 8) on the radioresistance of persistent antibody responses in the mouse, i.e., that the exposure of animals with ongoing IgE (and IgG) responses to lethal doses of X-rays sufficient to deplete B memory cells (26, 27) has little effect upon antibody titers in the ensuing month.

As the median life span of antibody-forming cells appears to be between 2 and 4 days (28, 29), these latter findings have been interpreted as evidence for the existence of long-lived IgE plasma cells (4, 25) similar to those originally identified by Miller (30) as being involved in long-term production of other classes of antibody. It should be noted that an earlier report (31) suggested that IgE-B cells may be differentially resistant to doses of X-rays below 400 R; however, more recent studies (4, 5, 8, 25) employing higher doses indicate that the sensitivities of primed IgE- and IgG-B cells are equivalent.

The present study provides fresh insight into these plasma cells at several levels, particularly the population involved in persistent IgE production. First, these cells are not restricted to mice, as they are equally evident in BN rats (Table 1). Second, as reported earlier in mice (8), long-lived plasma cells in rats are refractory to the effects of suppressor cells of the type which abrogate primary IgE responses (Fig. 2). Third, the use of a novel ELISA-based PFC assay has permitted the localization of the cells responsible for persistent IgE production in different lymphoid organs in these two species. As noted above, the bone marrow appears to be of major importance as a site for long-term IgG production (as noted in other studies reviewed in (32)), and also for IgE production, at least in the mouse (Table 2). By contrast, persistent IgE-producing cells in the rat are restricted almost entirely to the lymph node draining the site of initial antigen challenge, whereas those secreting IgG appear in bone marrow and spleen as well as in the draining node. The significance of these species differences is not presently clear, and may reflect

variations in plasma cell migration patterns or in the tissue distribution of regulatory T cells.

Previous studies on the induction of long-lived antibody-secreting cells in the mouse were restricted to the use of potent immunogen/adjuvant combinations in high-response animals (3-5, 8, 25). The experiments reported here indicate that more physiological modes of antigen exposure, including passive inhalation of an antigen aerosol (Table 1d) are sufficient to stimulate production of these cells.

It should also be noted that in several of the present experiments, lethal X-irradiation effected a reduction in ongoing antibody production of up to 50%. While this may be due in part to indirect toxic sequelae of radiation treatment, it may also indicate a significant underlying contribution from continued recruitment of lymphocytes into active antibody synthesis; the recent discovery that irradiation facilitates the expression of IgE-enhancing factor activity (33, 34) may be relevant to this process. The coexistence of both pathways of persistent antibody production, the latter susceptible to T-cell regulation and the former not (as shown Fig. 2), may underlie the variable success of conventional immunotherapy in the treatment of human atopy. This possibility is under continuing investigation.

ACKNOWLEDGMENTS

This work is supported by the Princess Margaret Children's Medical Research Foundation, the Asthma Foundation of Western Australia, and TVW Telethon Foundation. This is Publication 184 of the Clinical Immunology Research Unit of the Princess Margaret Children's Medical Research Foundation of Western Australia.

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