

CONCOMITANT IMMUNOGLOBULIN E AND IMMUNOGLOBULIN G1 FORMATION IN *Nippostrongylus brasiliensis*-INFECTED MICE¹

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The immunoglobulin (IgE, IgG subclass, and IgM formation in vivo and in vitro were measured by radioimmunoassays in normal and immunodeficient mice infected with the parasite *Nippostrongylus brasiliensis* (Nbr). High IgE responder mice had higher IgE serum levels than low responder mice both before (100 to 250 vs 2 to 20 ng of IgE/ml) and after (17,000 to 27,000 vs 1,000 to 5,000 ng of IgE/ml) infection. In vitro, mesenteric lymph node cells formed 5- to 10-fold more IgE than the spleen cells. Nu/nu mice had <1 ng before and 5 ng of IgE/ml serum after Nbr infection; no IgE was detectable in cell supernatants. There was no difference in the in vivo and in vitro IgE formation between C57BL/6 and C57BL/6-beige mice. Both in vivo and in vitro Nbr-infected *Xid*⁺ mice (CBA/N; (CBA/N × BALB/c) F₁ male) formed approximately twice the amount of IgE than *Xid*⁻ mice. Concomitant with the rise of the IgE serum levels, the IgG1 serum level increased by 100 to 1,500 µg/ml over preinfection levels in all strains except nu/nu⁺ mice. The IgM serum level increased by 100 to 500 µg/ml. In contrast, the IgG2a and IgG2b levels decreased in most strains, and the IgG3 levels remained unchanged. Nu/nu⁺ mice showed an increase of all IgG subclasses after Nbr infection. The antibodies to an Nbr extract were predominantly of IgM and IgG1, except in SJL mice which also had IgG2b and nude mice which only had IgM antibodies.

The concomitant increase of IgE and IgG1 formation in all but the nude strains suggests that the regulation of these two Ig isotypes may be linked via regulatory T helper cells, which corroborates recent data on the simultaneous induction of IgG1 and IgE synthesis in vitro by the B cell stimulatory factor 1.

The mechanism controlling antibody formation of the immunoglobulin (Ig)E³ isotype is of particular interest because of its implication in the pathology of allergic

diseases in man (1). In the past, it has been shown that IgE formation is controlled by genetic factors which involve both major histocompatibility-linked and nonlinked loci (2-6). Different strains of mice can be divided into high and low IgE responder strains according to predictable IgE antibody responses. Environmental factors also influence IgE production. Certain bacterial products (7) and infections with helminth parasites (8) enhance IgE antibody production. On a cellular level, it was established that the initiation of IgE formation is highly dependent on T helper cells (9-11). IgE-suppressive roles have been characterized for radiosensitive T suppressor cells (12, 13) and T cells forming IgE-binding factors (14, 15).

Recently, it has been shown that T helper cells can be divided in two subtypes on the basis of their lymphokine formation (16). Coffman et al. (17, 18) showed that one type of T helper cell produces B cell stimulatory factor-1 (BSF-1, also called interleukin-4) that enhances IgE formation by lipopolysaccharide-activated normal murine B cells. The other T helper subset forms interferon-γ (IFN-γ). Inasmuch as IFN-γ abrogates the BSF-1-induced IgE synthesis in vitro (17), it was hypothesized that the IgE regulation may be dependent on the activities of these T helper cell subsets. However, the exact mechanism by which T cells control the IgE production in vivo is not fully understood. Furthermore, natural killer (NK) cells which also form lymphokines including B cell growth factor and IFN-γ (19) also have been reported to affect IgE formation (20).

To gain more insight into the role of different lymphocyte subtypes in the regulation of IgE synthesis, we studied the IgE formation occurring after *Nippostrongylus brasiliensis* (Nbr) infection in different strains of mice having genetic defects in certain lymphocyte populations. The strains of mice tested included mice having the athymic nude mutation, the beige mutation which affects NK cell function (21), and the *Xid* mutation which is a pleiotropic defect affecting both B (22) and T (23) cell functions.

In the past, IgE formation in mice was usually measured by the semiquantitative method of passive cutaneous skin reaction in rats (11-13). This method did not allow us to study the increase of polyclonal IgE after parasitic infection compared with specific IgE. In rats it has been shown that Nbr infection not only induces parasite-specific IgE but also large quantities of non-antiparasite polyclonal IgE (8). Therefore, we developed a radioimmunoassay (RIA) to quantitate and compare the serum levels of IgE in different strains of mice. The IgG serum levels of all four subclasses were also determined to investigate whether IgE and IgG subclasses are coor-

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³ Abbreviations used in this paper: MLN, mesenteric lymph node; Nbr, *Nippostrongylus brasiliensis*; NK, natural killer; BSF-1, B cell stimulatory factor 1 (interleukin 4); IFN-γ, interferon-γ.

dinately regulated *in vivo*. Furthermore, we cultured the spleen and mesenteric lymph node (MLN) cells to determine whether IgE and IgG formation *in vitro* correlates to the IgE and IgG serum levels.

MATERIALS AND METHODS

Mice. From 8- to 10-wk-old BALB/c, BALB/c, nu/nu, C57BL/6, C57BL/6 nu/nu, C3Heb/FeJ, SJL, CBA/J, CBA/N, and (CBA/N × BALB/c)_{F1} were obtained from the Scripps Clinic Research Institute breeding colony. Blood was obtained at time of death or from retro-orbital venous plexus from anesthetized mice.

Parasites. Nbr parasites that had been adapted to mice (24) were kindly provided by Dr. Joseph Urban, U.S. Department of Agriculture, Animal Parasitology Institute, Beltsville, MD. The parasites were maintained in Lewis rats, and groups of three to four mice were injected s.c. with 400 third stage larvae. Nbr antigen extract was prepared as described by Ogilvie (25) and used to coat the RIA plates.

Mouse IgE. Two monoclonal mouse IgE anti-dinitrophenol (DNP) antibodies, one (H1-DNP-ε-26) provided by Dr. N. Klinman (26), Scripps Clinic, and one (IGEL a2) obtained from American Type Culture Collection, Rockville, MD, (27) were immunosorbent purified from ascites of mice bearing the hybridomas on a Sepharose 4B column to which DNP-bovine serum albumin was coupled. The antibodies were eluted from the column with 0.1 M DNP, pH 7.4, dialyzed overnight against phosphate-buffered 0.15 M NaCl and the remaining DNP removed by passage of the protein through Dowex-1 columns equilibrated with borate-buffered 0.015 M NaCl, pH 7.5. The concentration of purified IgE was determined by OD at 280 nm, employing extinction coefficient of $E_{280}^{1\%} = 15$. Monoclonal IgE with specificities for arsonate (28) and ovalbumin (29) were kindly provided by Dr. A. Nisonoff, Brandeis University, Waltham, MA, and Dr. Maurice Scheetz, Eli Lilly Company, Indianapolis, IN, respectively.

Myeloma proteins. Murine myeloma proteins of classes IgG1, (MOPC21), IgG2a (PC10), IgG2b (MOPC 195), IgG3 (J606), IgM (MOPC 183), and IgA (TEPC 15) were obtained from Bionetics Laboratories, Kensington, MD.

RIA. The IgE levels in the serum and culture fluids of the different strains of mice were determined by a 96-well polyvinyl plate RIA described previously for human IgE (30) except for use of purified goat antibodies specific for mouse ε-chains. A goat was immunized with the monoclonal mouse IgE antitritonphenol H1 DNP-ε-26 (26), the antiserum was absorbed with normal mouse serum containing <100 ng IgE/ml, and the antibodies purified by using a Sepharose 4B column to which the IgE anti-OVA was coupled. The mouse sera were tested for IgE levels at three serial dilutions to ensure that the slopes of the serial dilutions paralleled those of the monoclonal IgE standards. The lowest IgE concentration that could be measured reproducibly was 0.4 ng of IgE/ml.

The serum levels of the four IgG subclasses and IgM were determined by using the same type of RIA except that purified goat antibodies specific for the IgG subclasses and IgM obtained from Southern Biotechnology Associates, Birmingham, AL, were used. Myeloma proteins of the appropriate subclasses served as standards. No cross-reaction of the purified antibodies with IgG of another subclass or IgE was detectable. The lowest concentrations that could be measured accurately varied from 1 to 3 ng/ml for the different IgG subclasses. IgA serum levels were measured by radial immunodiffusion using a goat antiserum to mouse IgA obtained from Bionetics Laboratories, Kensington, MD. Differences in Ig serum levels of groups of four individual mice were analyzed for significance by the Student's *t*-test.

Antibody activity to an Nbr extract was studied by an RIA involving the same reagents and methods, except that the plates were coated for 1 hr at room temperature with 100 μl of a 1/20 dilution of an Nbr worm extract. The antisera were tested at a dilution of 1/50, the optimal dilutions of both antigen and antisera having been determined in preliminary experiments.

Cell preparations. Single cell suspensions were prepared in a sterile manner from spleen and MLN by passage of the cells through a stainless steel screen. The cells were centrifuged at low speed and the erythrocytes lysed by incubation of the cells for 20 sec in distilled water followed by three washes with the culture medium which consisted of RPMI 1640 medium containing 10% fetal bovine serum, 5×10^{-5} M 2-mercaptoethanol, 2 mM glutamine, and antibiotics. The cells were cultured at 5×10^6 cells/ml in 24 flat-bottomed well plates (Linbro, Flow Labs, McLean, VA). After 10 days the culture supernatants were harvested and stored at -20°C before being analyzed for Ig concentration. Cycloheximide at 100 μg/ml was added to control cultures to determine the *in vivo* preformed cell-associated IgE or IgG. The quantities of IgE or IgG released in the presence of

cycloheximide were subtracted from the concentration of the experimental cultures.

RESULTS

IgE and IgG RIA. Standard curves for IgE and logarithm of counts bound of serial dilutions of normal or Nbr-infected mouse sera are shown in Figure 1. The purified anti-ε-chain-specific antibodies reacted equally with the monoclonal IgE used for immunization and an unrelated IgE monoclonal antibody, indicating that they did not contain antibodies to idiotype determinants of the IgE antibody that was used for immunization. The slopes of serial dilutions of sera from normal and Nbr-infected mice of all strains tested were identical to those of the isolated monoclonal IgE.

The serum levels of the four different IgG subclasses were measured with commercially available purified goat antibodies to IgG subclasses. The antibodies reacted specifically with myeloma proteins of the appropriate subclass. However, most of the antibodies to the IgG2a subclass appeared to be directed to IgG-1^a allotypic determinants (31) because serial dilutions of serum from C57BL/6 and SJL mice which are of IgG-1^b allotype showed a slope different from serial dilutions of the BALB/c myeloma proteins PC10 and from sera of BALB/c, CBA/J, and C3Heb/FeJ mice which are of IgG-1^a allotype. Because no isolated IgG2a of IgG-1^b allotype was available as a standard, no accurate IgG2a measurements could be made in C57BL/6 and SJL mice.

IgE and IgG serum levels in normal and nude mouse strains before and after Nbr infection. To study the effect of Nbr infection on the serum level of the different Ig isotypes, BALB/c mice were injected in preliminary experiments with 400 Nbr larvae and the Ig serum levels determined over a 2-mo period. The IgM levels increased about twofold within 2 wk after infection, whereas no significant changes in the IgA levels were detected. In contrast, the IgE serum level increased >100 fold, reaching a peak at approximately 14 days after infection. Similarly, the changes in IgG subclass levels were maximal 2 wk after infection. Therefore, in subsequent experiments, the IgE and IgG subclass levels were measured before and 2 wk after the Nbr infection. In some experiments, sera as well as spleen and MLN cells from three mice were pooled for analyses, whereas in others the Ig

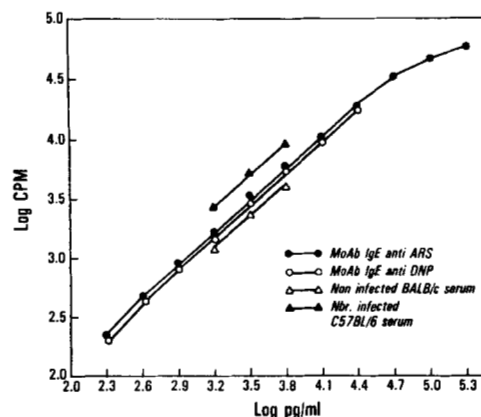


Figure 1. RIA for mouse IgE. Logarithm of counts bound to dilutions of the IgE anti-DNP H1 (DNP-ε-26) that was used for immunization, IgE antiarsonate (ARS) that was used for the standard (28), and twofold dilutions of normal BALB/c serum and serum of C57BL/6 Nbr-infected mice.

serum concentrations were quantitated individually in three mice for statistical analyses of the differences.

The IgE, IgG subclass, and IgM serum levels of normal mice before and after Nbr infection are shown in Table I. The IgE high responder strains (BALB/c, CBA/J, C3HeB/FeJ) had significantly higher IgE serum levels than the IgE low responder strains (C57BL/6, SJL) both before and after Nbr infection. Both IgE high and low responder strains showed an increase in the IgG1 levels. In contrast, the IgG2a and IgG2b levels decreased after infection in all strains except SJL. As described above, the IgG2a levels could not be measured accurately in strains of the IgG2a Igh-1^b allotype because most anti-IgG2a antibodies were directed to the Igh-1^a allotype. However, the relative counts bound in the IgG2a RIA decreased after infection in C57BL/6 mice and increased in SJL mice. The IgG3 serum levels did not change markedly after Nbr infection, whereas the IgM serum levels increased in all strains. The changes of the IgE, IgG1, IgG2a, IgG2b, and IgM subclass levels were statistically significant ($p < 0.05$ or less) as measured in two separate experiments.

The responses of BALB/c and C57BL/6 nu/nu strains are shown in Table II. Both strains had <1 ng of IgE/ml serum before Nbr infection and 4 to 6 ng of IgE/ml after infection. The IgG subclass levels were low compared with normal BALB/c and C57BL/6 mice (Table I). The concentrations of all IgG subclasses and IgM increased after Nbr infection, IgG1 and IgG2a showing the largest increase.

IgE and IgG serum levels in mice carrying the beige or Xid mutations. Because the beige mutation is associated with an NK cell defect, the IgE and IgG subclass response to Nbr infection of normal C57BL/6 mice was compared with C57BL/6-beige mice. As shown in Table III, Nbr infection induced a large and similar increase of the IgE and IgG1 serum level in both strains. The IgG2b level decreased in C57BL/6 and increased in C57BL/6-beige mice, whereas the IgG3 levels were low and did not show large changes. The IgM levels increased slightly more in the C57BL/6-beige than in their normal counterparts.

To determine whether the Xid mutation affects IgE and IgG subclass responses to Nbr infection, CBA/N and

(CBA/N $\times$ BALB/c) F₁ male mice were studied and compared with CBA/J (Table I) and (CBA/N $\times$ BALB/c) F₁ female mice, respectively (Table IV). The Xid mice had IgE preinfection levels similar to normal IgE high responder strains. After infection, they formed approximately twice as much IgE as IgE high responder strains. Xid mice had a lower IgG1 level which increased about threefold after infection, whereas the IgG2a and IgG2b levels decreased. As a characteristic of the mutation, the IgG3 and IgM levels were very low (32) and did not change after infection. In two additional experiments F₁ males formed approximately twice as much IgE as the F₁ females ($p < 0.01$).

Isotype distribution of antibodies to Nbr extract. To determine whether the changes in the Ig serum levels paralleled the antibody production to Nbr, the sera of the mice shown in Tables I through IV were tested in a semiquantitative manner for the Ig isotype of antibodies reacting with an Nbr worm extract. As shown in Table V, most of the anti-Nbr activity was of IgM and IgG1 class in all strains, except SJL mice which had the largest activity in the IgG2b subclass. A small antibody formation of the other classes may not have been detected because of limited amounts of antigen in the assay and competition by the large quantities of IgM and IgG1 antibodies. To determine whether IgE anti-Nbr antibodies were indeed produced, the IgE was purified with an anti-IgE immunosorbent column from 10 ml of Nbr-infected BALB/c serum, diluted to the original 10-ml-serum volume, and tested at a 1/50 dilution. This purified IgE preparation bound 2200 cpm compared with 180 cpm for serum pool at the same dilution, suggesting that competition for the limited amount of antigen resulted in the low cpm-bound IgE. Still, Table V shows that most of the anti-Nbr antibodies were of classes IgM and IgG1.

Of the immunodeficient mice, C57BL/6-beige mice showed similar IgM and IgG2b activities to C57BL/6 but formed less IgG1 anti-Nbr. C57BL/6 nu/nu mice showed only IgM activity. (CBA/N $\times$ BALB/c)F₁ males had low IgM and IgG3 titers compared with the F₁ females; the IgE activity was slightly higher in the F₁ males than females.

Spontaneous IgE and IgG subclass formation in vitro by spleen and MLN cells of different strains of mice. To determine whether the spontaneous Ig synthesis in vitro

TABLE I
IgE, IgG subclass, and IgM serum levels in high and low IgE responder mouse strains before and 14 days after Nbr infection

Strain	Infection	IgE ng/ml ^a	IgG1	IgG2a	IgG2b μg/ml ^b	IgG3	IgM
High responder							
	BALB/c						
	-	145 ± 46	349 ± 52	720 ± 35	202 ± 27	139 ± 53	450
	+	17,072 ± 2,682	1,275 ± 316	369 ± 158	160 ± 45	242 ± 88	900
	CBA/J						
Low responder							
	-	158	225	470	158	24	448
	+	27,000	600	316	110	23	570
	C3HeB/FeJ						
	-	230	500	380	178	180	ND ^b
	+	17,000	630	250	125	160	ND
Low responder							
	C57BL/6						
	-	11 ± 7	466 ± 288	ND	617 ± 47	31 ± 18	407
	+	2,060 ± 753	1,475 ± 386	ND	475 ± 139	32 ± 11	764
	SJL						
	-	11	630	ND	300	71	501
	+	5,000	2,100	ND	350	90	1,091

^a Mean ± SD of three mice or pool of sera from three mice.

^b ND, not determined.

TABLE II

IgE, IgG subclass, and IgM serum levels in BALB/c nu/nu and C57BL/6 nu/nu mice before and 14 days after Nbr infection

Strain	Infection	IgE ^a ng/ml	IgG1	IgG2a	IgG2b μg/ml	IgG3	IgM
BALB/c nu/nu	-	<1	33	120	50	35	500
	+	6	88	340	89	63	633
C57BL/6 nu/nu	-	<1	16	ND ^b	158	62	350
	+	4	50	ND	168	94	373

^a Ig concentrations in pool of sera from three mice.

^b ND, not determined.

TABLE III

IgE, IgG subclass, and IgM serum levels in mice carrying the beige mutation before and 14 days after Nbr infection

Strain	Infection	IgE ^a ng/ml	IgG1	IgG2a	IgG2b μg/ml	IgG3	IgM
C57BL/6	-	16	400	ND ^b	630	50	442
	+	1120	1100	ND	430	45	1328
C57BL/6-beige	-	12	120	ND	151	14	335
	+	1700	1270	ND	342	25	2098

^a Ig levels in pool of sera from three mice.

^b ND, not determined.

TABLE IV

IgE, IgG subclass, and IgM serum levels in mice carrying the XID gene before and 14 days after Nbr infection

Strain	Infection	IgE ^a ng/ml	IgG1	IgG2a	IgG2b μg/ml	IgG3	IgM
CBA/N	-	115	88	890	150	4	45
	+	40,000	250	150	69	1	50
(CBA/N × BALB/c) F ₁ males	-	343	210	977	317	30	20
	+	36,150	684	94	61	12	26
F ₁ females	-	314	517	900	933	140	378
	+	15,275	2,194	400	138	150	523

^a Ig concentrations in pools of sera from three mice.

of the different isotypes correlates to the serum levels, the spleen and MLN cells of the mice shown in Tables I to IV were cultured for 10 days and the Ig levels determined in the cell supernatants. Ig levels of cells cultured in presence of 100 μg of cycloheximide/ml were subtracted to correct for in vivo preformed Ig released during the culture period. As shown in Table VI, spleen and MLN cells of uninfected mice of all strains formed <1 ng of IgE/ml/5 × 10⁶ cells. After Nbr infection, significant IgE levels were detected in supernatants of both spleen and MLN cells of all strains tested except the nude strains. MLN cells formed 2- to 10-fold more IgE than the spleen cells, despite the fact that they contained only 30% B cells compared with 60% for the spleen cells (data not shown). Cells from IgE high responder strains formed

more IgE than those from low responder strains. There was no difference in IgE formation in vitro between C57BL/6 and C57BL/6-beige mice. Cells from CBA/N mice formed the most IgE in vitro. Although MLN cells from (CBA/N × BALB/c)F₁ male mice formed only slightly more IgE than the females, the males formed 3 times more IgE than the females when calculated on the percentage of B cells in the preparation, because the males had only one-third of the percentage of B cells compared with the females.

In all strains of mice, the IgG1 synthesis in vitro by both spleen and MLN cells increased after Nbr infection. The IgG2b synthesis usually also increased, even in the mice that showed a decrease of the IgG2a and IgG2b serum levels. Except for the nude mice, the IgG1 synthesis in vitro far exceeded that of the IgG2b synthesis, particularly in the MLN of Nbr-infected mice.

DISCUSSION

Infection of mice with the parasite Nbr induced, in addition to IgM, a concomitant increase of the IgG1 and IgE serum levels in all strains tested except athymic nude mice. In contrast, the IgG2a and IgG2b levels decreased, and the IgG3 concentrations remained relatively constant. The antibody activity to an Nbr worm antigen preparation was predominantly of the IgG1 and IgM isotype. The IgE antiparasite activity measured by RIA was low, usually lower than the IgG2a and IgG2b antibody activities. The reason for this is most likely the difference between the absolute and relative increases of the IgG1, IgM, and IgE formation. Although the IgE serum levels rose >100 fold over the preinfection levels, they increased by only 10 to 40 μg/ml compared with 130 to 1500 μg/ml for IgG1 and IgM. Inasmuch as the majority of Nbr-induced IgE is not IgE antiparasite but "nonspecific" polyclonal IgE (8), most of the parasite-induced IgG1 and IgM is presumably also not antiparasite antibody. Assuming that the percentage of antiparasite activities in the three Ig classes is similar, it is not surprising the IgE activity was low because much more IgG1 and IgM than IgE was formed after the Nbr infection. In the past, Nbr infection has been used specifically as a model for studying IgE formation (8, 13, 24). However, in terms of quantity, Nbr infection in mice induces 25- to >100-fold more IgG1 and IgM than IgE. Although not emphasized in previous studies of changes in Ig serum levels of Nbr-infected rats (33), the IgG1 serum level also increased substantially from a mean of 4.5 to 12 mg IgG1/ml. This indicates that IgG1 is the major Ig isotype formed in

TABLE V
Antibodies of different classes reacting with Nbr worm extract in sera of different mouse strains

Strains	IgE	IgG1	IgG2a	IgG2b cpm ^a	IgG3	IgM
BALB/c	67 ± 18	6,540 ± 1,145	88	209 ± 63	29 ± 19	14,427 ± 946
CBA/J	81	2,024	508	631	47	13,650
SJL	118	8,894	ND ^b	16,429	1,025	12,009
C57BL/6	27 ± 17	3,939 ± 1,118	ND	306 ± 144	28 ± 25	7,974 ± 2,325
C57BL/6-beige	0	879	ND	800	0	11,144
C57BL/6 nu/nu (CBA/N × BALB/c)	0	0	ND	0	0	8,299
F ₁ males	684	2,527	881	757	0	3,581
F ₁ females	402	3,255	521	325	208	28,452

^a Number cpm at serum dilution of 1/50, mean ± SD of three mice or serum pools of three mice, background count to wells containing no Nbr which varied from 100 to 300 cpm were subtracted. Counts bound in preimmune sera were within the range of the background of uncoated wells.

^b ND, not determined.

TABLE VI
IgE and IgG subclass concentrations in culture supernatants of spleen (S) and MLN cells of normal and Nbr-infected mice

Cell Source		ng/ml/5 × 10 ⁶ cells							
		IgE		IgG1		IgG2b		IgG3	
		N	I	N	I	N	I	N	I
High responders ^a									
BALB/c	S	<1	13	565	866	70	223	400	496
	MLN	<1	115	448	7000	56	178	136	280
CBA/J	S	<1	18	106	603	420	858	98	90
	MLN	<1	67	40	2510	126	580	28	82
Low responders									
C57BL/6	S	<1	2	145	252	112	89	125	35
	MLN	<1	11	140	895	100	250	94	565
SJL	S	<1	2	120	802	79	160	29	47
	MLN	<1	6	772	1972	272	278	193	114
Nude mice									
BALB/c nu/nu	S	<1	<1	<5	11	<5	<5	12	<8
	MLN	<1	<1	<10	26	<10	70	<10	93
C57BL/6 nu/nu	S	<1	<1	96	418	120	784	160	556
	MLN	<1	<1	40	436	248	1320	240	>2000
Beige mutation									
C57BL/6-beige	S	<1	4	86	583	50	468	44	125
	MLN	<1	4	110	1244	120	296	316	316
Xid mutation									
CBA/N	S	<1	73	4	115	67	18	<2	<2
	MLN	<1	270	<30	760	42	80	<2	10
F ₁ male ^b	S	<1	42	156	210	120	<50	80	<2
	MLN	<1	138	52	257	<50	<10	<2	<2
F ₁ female ^b	S	<1	47	156	757	<50	100	50	125
	MLN	<1	116	483	1358	140	<50	632	250

^a Pools of cells from two to five mice. N = normal mice. I = 14 days Nbr-infected mice.

^b (CBA/N × BALB/c)F₁ male and female.

response to Nbr infection in rodents.

Mitchell and his colleagues (34, 35) showed that chronic infections in mice with certain parasites, such as *Mesocostoides corti*, induce very high IgG1 serum levels without significant changes in the levels of IgG subclasses or IgA. The IgE serum levels were not determined in these experiments. Recently, Abraham and Teale (36) reported that infection of mice with *M. corti* induces almost entirely IgG1 antiparasite or IgG1 anti-DNP antibodies to haptened *M. corti* parasites. Because these authors measured only antibody activity, the assays may not have been sensitive enough to detect a small percentage of IgE antiparasite antibodies, particularly because the mice were studied 6 mo after infection when they have very large amounts of IgG1 antibodies that compete effectively for antigen. Preliminary experiments performed in our laboratory on the serum levels of *M. corti*-infected mice showed that IgE rises concomitantly with IgG1, indicating that polyclonal IgG1 formation also parallels IgE formation in *M. corti* infection.

The concomitant increase of IgG1 and IgE in Nbr-infected mice suggests that the regulation of these two isotypes may be linked. Recent observations by Coffman et al. (17, 18) on the effect of the murine lymphokine BSF-1 (or interleukin-4) on Ig synthesis in vitro support this hypothesis. These authors showed that BSF-1 induces normal lipopolysaccharide-stimulated B cells to secrete not only IgG1, as had been shown before (37), but also IgE. In absolute quantities, BSF-1 induced much more IgG1 than IgE, although the relative increase was most striking for IgE. These data corroborate our in vivo findings on the effect of Nbr infection. Mosmann et al. (16) further showed that T helper cell clones can be divided into two categories based on the profile of lymphokine secretion. One type of T helper cell clones, called T_{H1}, forms interleukin 2 and INF- γ , whereas the other,

called T_{H2}, secretes BSF-1 but no interleukin 2 and INF- γ . Because INF- γ abolishes IgG1 and IgE secretion in vitro, Coffman et al. (17) hypothesized that IgE formation may be regulated by a balance between the two T helper cell subsets. Although it is unknown how the Nbr parasite induces IgG1 and IgE, the parallel increase of these two isotypes provides in vivo evidence that IgE and IgG1 may be coregulated, perhaps through T_{H2} cells that produce BSF-1. Indeed, recent experiments by Finkelman et al. (38) showed that injection of a rat monoclonal antibody to murine BSF-1 inhibits IgE formation. In contrast, it had no significant effect on the IgG1 level. Although these experiments could be interpreted to indicate that IgG1 and IgE formation are not linked in vivo, it does not rule out a concordant regulation, if IgE formation is quantitatively more dependent on BSF-1. The concomitant increase of IgE and IgG1 after injection of mice with goat anti-IgD (39, 40), a stimulus quite different from parasitic infections, also suggests a regulatory linkage between IgE and IgG1. These experiments do not exclude the possibility that IgE or IgG1 may also be induced by mechanisms not involving BSF-1.

The decrease of the IgG2a and IgG2b after Nbr infection was most likely the result of a low IgG2a and IgG2b production combined with an increased IgG catabolism resulting from the large IgG1 production. It has been shown that IgG of different subclasses uses the same catabolic pathway and that an increase of one IgG subclass induces an increase of the catabolism of all IgG subclasses (41). The decrease of the IgG2a and IgG2b serum levels could also have been the result of specific immunosuppression by the parasite. However, this is unlikely for three reasons. First, the sera of most infected mice showed specific IgG2a and IgG2b antiparasite activity, although at low titer, indicating formation of new IgG2a and IgG2b during infection. Second, the sponta-

neous IgG2a and IgG2b synthesis in vitro was increased rather than decreased after Nbr infection. Spontaneous Ig synthesis in vitro most likely reflects synthesis by in vivo-activated cells. If the parasites suppressed differentiation of B cells to secreting plasma cells, then an increase of spontaneous IgG2a and IgG2b synthesis would not have been detected. Third, in SJL and C57BL/6-beige mice, IgG2b serum levels increased after Nbr infection, with SJL mice showing the highest anti-Nbr activity in the IgG2b class. If the parasites would have suppressed formation of IgG2a and IgG2b, it is unlikely that this would have occurred in only a fraction of the mouse strains tested. For all these reasons, specific induction rather than suppression is most likely the mechanism responsible for the predominant formation of IgG1 and IgE in Nbr-infected mice.

The increase of IgE and IgG1 synthesis in vitro after Nbr infection was much greater in MLN than spleen cell cultures. Apparently, the stimulatory effect of the parasite on Ig synthesis of these isotypes was localized anatomically to the site of infection, at least for the 14 days time point. The difference between MLN and spleen becomes even twofold larger when the Ig synthesis is calculated on the basis of the percentages of B lymphocytes because the MLN cells contained only half as many B cells as compared with spleen cells. This finding suggests that a large portion of the total IgE and IgG1 was synthesized at the site of infection. The predominant IgE synthesis by MLN cells indicates that IgE synthesis in vitro cannot be expected with lymphocytes isolated from all anatomic sites. Perhaps this is the reason why IgE synthesis in vitro by human peripheral blood lymphocytes usually does not correlate to the IgE serum levels of the cell donors (30, 42).

It has been reported that human NK cells suppress IgE synthesis in vitro (20), suggesting that these cells may play a down-regulatory role in IgE formation. Furthermore, NK cells produce IFN- γ (19) and IFN- γ inhibits BSF-1-induced IgE formation in vitro (18). To test whether NK cells may play a suppressive role in IgE formation in vivo, we compared the IgE formation between C57BL/6 and C57BL/6-beige mice, the latter having a defect in NK cell activity (21). Although the mice carrying the beige mutation showed a sixfold lower IgG1 anti-Nbr antibody response, the increases of the total IgE and IgG1 were similar to the normal C57BL/6 mice, indicating that the NK cell defect in beige mice is not affecting IgE regulation. Because NK cells are heterogenous, this does not exclude a role of NK cells in IgE regulation.

(CBA/N $\times$ BALB/c) F_1 male mice carrying the *Xid* mutation formed significantly more IgE than the *Xid*⁻ F_1 females, indicating that the *Xid* gene was responsible for the high IgE production. The *Xid* mutation is pleiotropic resulting in a B cell defect with low percentages of splenic B cells, low IgM and IgG3 production, and absence of T-independent and T-dependent type 2 antigen responses (22, 32). Although the high IgE formation in *Xid*⁺ mice could be due to the presence of increased numbers of IgE-committed B cells, it appears more likely that an *Xid*-associated T cell abnormality is responsible for the high IgE production. In adoptive transfer experiments, Teale (23) showed that B cells from *Xid*⁺ F_1 male mice produce normal IgG3 and antiphosphorylcholine responses in F_1 female mice. Furthermore, the enhanced IgE antiphos-

phorylcholine response by B cells from male mice was reduced to a normal IgE response in the F_1 female environment. Assuming that the extent of an IgE response is the result of T cell subsets, *Xid*⁺ mice may have fewer T_{H1} or more T_{H2} helper cells than normal mice, thus favoring an enhanced IgE production. When reagents become available to enumerate T_{H1} and T_{H2} helper cells, this possibility can be investigated.

Based on antigen-specific IgE responses measured semiquantitatively by the passive cutaneous anaphylactic reaction in rats, mice have been divided into IgE high and low responder strains (2-6, 13). By measuring the IgE serum concentration with an IgE-specific RIA, we showed that IgE high responder mice have significantly higher IgE serum levels than low responder strains both before and after Nbr infection. The high responder strains apparently formed more IgE than the low responder strains to environmental antigens and to Nbr parasites.

In conclusion, it was shown that in vivo IgE formation in Nbr-infected mice is linked to IgG1 formation. Although it is not understood how the Nbr parasites induce the predominant IgE and IgG1 formation, the in vitro experiments of Coffman et al. (17) strongly suggest that both IgE and IgG1 formation is dependent on T helper cells forming BSF-1. Quantitation of BSF-1 production by MLN cells from normal and Nbr-infected mice analogous to those reported by Finkelman et al. (43) in anti-IgD-treated mice will have to be performed to test this hypothesis. The coupling of the regulation of an IgG subclass with IgE may also apply to human responses. It will be interesting to determine whether enhanced IgE formation in atopic patients or patients with immunodeficiencies such as Wiscott-Aldrich syndrome which resembles the *Xid* mutation in mice (44) is linked to enhanced formation of a particular IgG subclass, perhaps IgG4. Like rodent IgG1, IgG4 does not fix complement and has an anodal electrophoretic mobility (45). IgE- and IgG4-restricted antigen-specific responses have been reported in atopic (46) and parasitically infected patients (47). Quantitation of both specific antibodies and IgE and IgG subclass serum levels in man may demonstrate a concordant regulation of IgE formation with a particular IgG subclass.

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