

24243



Department of Microbiology and Immunology, University of Western Ontario, London, Ont., Canada

Effect of Anti-DNP IgG1- and IgG2a-Secreting Hybridomas in vivo on the Development of an Anti-DNP IgE Antibody Response in Mice

Key Words

IgG
Anti-DNP IgE
Monoclonal antibodies
Hybridomas

Abstract

We reported previously that CBA mice pretreated with dinitrophenyl-*Bordetella pertussis* (DNP-BP) conjugates exhibited sharply decreased anti-DNP IgE, and increased IgG2a antibodies following immunization with DNP-ovalbumin (DNP-OA) in alum. The objective of the present experiments was to determine whether the decrease in anti-DNP IgE was attributed to a regulatory effect exerted by IgG2a antibodies. Anti-DNP monoclonal antibodies (Mab) of the IgG1 or IgG2a isotype were passively transferred to mice, 24 h before a primary immunization with DNP-OA in alum. Anti-DNP IgE production was drastically suppressed in recipients of IgG1 but not of IgG2a Mab. Similar results were obtained when the Mab were endogenously produced by intraperitoneal implantation of anti-DNP-secreting hybridomas into (BALB/cxCBA)F1 (BCF1) mice. However, neither IgG1 nor IgG2a isotypes suppressed IgE antibody production if the hybridoma implantation took place 10 days after hapten priming. These results are, to our knowledge, the first to show a clear dissociation between the effect of either passively transferred or endogenously secreted IgG1 and IgG2a antibodies in their ability to inhibit a primary anti-hapten IgE antibody response.

Introduction

The induction of an IgE antibody response is strictly T cell-dependent and requires functionally active, antigen-specific CD4⁺ T helper (TH) cells [1]. CD4⁺ T lymphocytes maintained in vitro as long-term lines have been divided into two subpopulations with distinct functions and lymphokine profiles: TH-1 clones produce IL-2, IFN- γ and lymphotoxin, while TH-2 clones produce IL-4 and IL-5. IL-4 plays a crucial role in the growth and development of B lymphocytes, and promotes the immunoglobulin (Ig)

isotype switch from IgM to IgG1 and IgE. IFN- γ inhibits the B cell stimulatory effects of IL-4, and promotes the Ig H chain switch from IgM to IgG2a [2]. Similar functional relationships appear to exist in vivo between various subpopulations of CD4⁺ T helper cells and their lymphokines, in various mouse strains and humans, supporting the notion that they represent the physiological correlates of the in vitro-derived clones [3, 4].

Although *Bordetella pertussis* vaccine (BP) is used as an adjuvant for the selective enhancement of IgE antibody production [5], we reported recently that the immuniza-

tion of CBA mice with 2,4-dinitrophenyl (DNP)-conjugated BP (DNP-BP) failed to stimulate anti-DNP IgE antibodies, even when an additional adjuvant – alum – was present in the inoculum. Furthermore, pretreatment of mice with either 1 or 3 injections of 50 µg (10^9) DNP-BP followed 14 days later by immunization with 2 µg DNP-ovalbumin (DNP-OA) in alum, resulted in an accelerated but short-lived anti-DNP IgE response, followed a few days later by a sharp IgE decline. In contrast, pretreatment of mice with equivalent amounts of trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH) or DNP-*Ascaris suum* antigen followed by immunization with DNP-OA in alum resulted in a sustained anti-DNP IgE and IgG1 primary response which persisted for at least 21 days [6]. In addition to the sharp drop in anti-DNP IgE levels, pretreatment with DNP-BP, but not with TNP-KLH induced a selective increase in IgG2a anti-DNP antibodies. Both the IgE decrease and the IgG2a increase were abolished by treatment of the DNP-BP-immunized mice with a monoclonal antibody against murine IFN- γ . The results suggested that the antigen-specific and isotype selective modulation observed after DNP-BP administration might have been a consequence of the effects of IFN- γ released by a predominantly TH-1 lymphocyte subpopulation. However, at the time of priming with DNP-OA in alum, the serum of DNP-BP-pretreated mice had detectable levels of anti-DNP IgG2a antibodies, which may have been responsible for the IgE decline, possibly through an Fc γ receptor-mediated regulatory mechanism. The aim of the present experiments was to determine whether or not the presence of anti-DNP IgG2a antibodies could account for the sharp decline in IgE antibodies observed in DNP-BP-pretreated mice.

We approached this question in two ways: (1) by the passive administration of anti-DNP monoclonal antibodies, 24 h before priming with DNP-OA in alum, and (2) by the adoptive transfer (implantation) of hybridomas secreting either IgG1 or IgG2a anti-DNP antibodies, prior to DNP-OA immunization. This was done on the premise that mice harbouring the IgG2a hybridomas would contain at the time of DNP-OA immunization, amounts of anti-DNP antibodies equivalent to those detected after DNP-BP pretreatment, thus simulating the conditions required for the IgE decline. The results show that the anti-DNP monoclonal antibodies (Mab) of the IgG2a isotype failed to interfere with the normal progression of the anti-DNP IgE response, after either passive Mab transfer or hybridoma implantation. Profound and persistent suppression of the IgE response was observed only in the presence of anti-DNP IgG1.

Materials and Methods

Animals

CBA/J male and BALB/c female mice, 8–12 weeks old were purchased from Jackson Laboratories, Bar Harbor, Maine. (BALB/c $\times$ CBA)F1 (BCF1) mice were bred in our animal care facility. Rats were Sprague Dawley females, retired breeders (Charles River Canada, Ottawa, Ont.).

Antigens

DNP-OA, DNP₁₈-BSA, TNP₂-KLH and DNP-BP were prepared as previously described [6, 7]. 4-Hydroxy-3-nitro-5-iodo-phenylacetyl (NIP) was conjugated to OA (NIP₁₈-OA) from NIP-succinamide (Biosearch Laboratories, San Rafael, Calif.) according to Weinberger et al. [8]. The subscripts refer to the average number of moles of hapten/mol of carrier, assuming a molecular weight of 40 kDa for OA, 72 kDa for BSA, and 800 kDa for the dissociated form of KLH. In case of DNP-BP, there were 6×10^6 groups/BP cell.

Hybridomas

For the construction of anti-DNP IgG1-secreting hybridomas, CBA mice were primed with 100 µg DNP-OA in complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Mich.), in 0.1-ml volumes divided among 4 subcutaneous (s.c.) sites. A booster injection of 100 µg DNP-OA in isotonic saline was given i.v. 14 days later. For anti-DNP IgG2a-secreting hybridomas, CBA mice were injected with 2×10^9 (100 µg) DNP-BP in CFA, and boosted 28 days later with the same amount of DNP-BP in saline, i.v. For the construction of anti-NIP-secreting hybridomas, CBA mice were primed and boosted with 100 µg NIP-OA as described above for DNP-OA. In all cases, the fusions were carried out 3 days after the last antigen injection. The non-secreting BALB/c mouse myeloma line SP2/0-Ag14 [9] obtained from the American Type Culture Collection (Rockville, Md.), and the spleen cells obtained from the immunized mice were fused in the presence of 40% polyethylene glycol (PEG)-1000 (BDH, Toronto, Ont.), essentially according to Kohler and Milstein [10]. After selection in HAT medium, the appropriate antibody-secreting hybridomas were cloned twice at 0.5 cells/well, by the limiting dilution technique. The clones were propagated either in vitro, in Dulbecco's modified essential medium (DMEM) (Gibco, Grand Island, N.Y.), supplemented with 10% fetal calf serum, or as ascites, by passage through mice that were injected intraperitoneally (i.p.), 7–60 days previously, with 0.5 ml 2,6,10,14-tetramethyl decanoic acid (Pristane) (Pfaltz & Bauer, Stamford, Conn.) [11]. The Mab used in the present study were obtained from ascites. The following hybridomas were used: anti-DNP clone AC2D12 (IgG1, k), anti-DNP IgG2a clone B4D4 (IgG2a, k), and an uncloned anti-NIP hybridoma, AH7, which secreted both IgG1 and IgG2a, k molecules.

Immunizations

For IgE antibody production, groups of 4–6 mice were immunized by a single i.p. injection of 2 µg DNP-OA in alum, in a volume of 0.5 ml. When indicated under 'Results', pretreatment consisted of 3 i.p. injections of 10^9 (50 µg) DNP-BP or 50 µg TNP-KLH, in isotonic saline, on days –16, –14 and –12. DNP-OA in alum was administered on day 0. Mice were bled by cardiac puncture, and as a rule, a maximum of 250 µl of blood was taken from each mouse in order to avoid exposing the mice to too much stress. For this reason, unless otherwise indicated in the 'Results', only serum pools were tested.

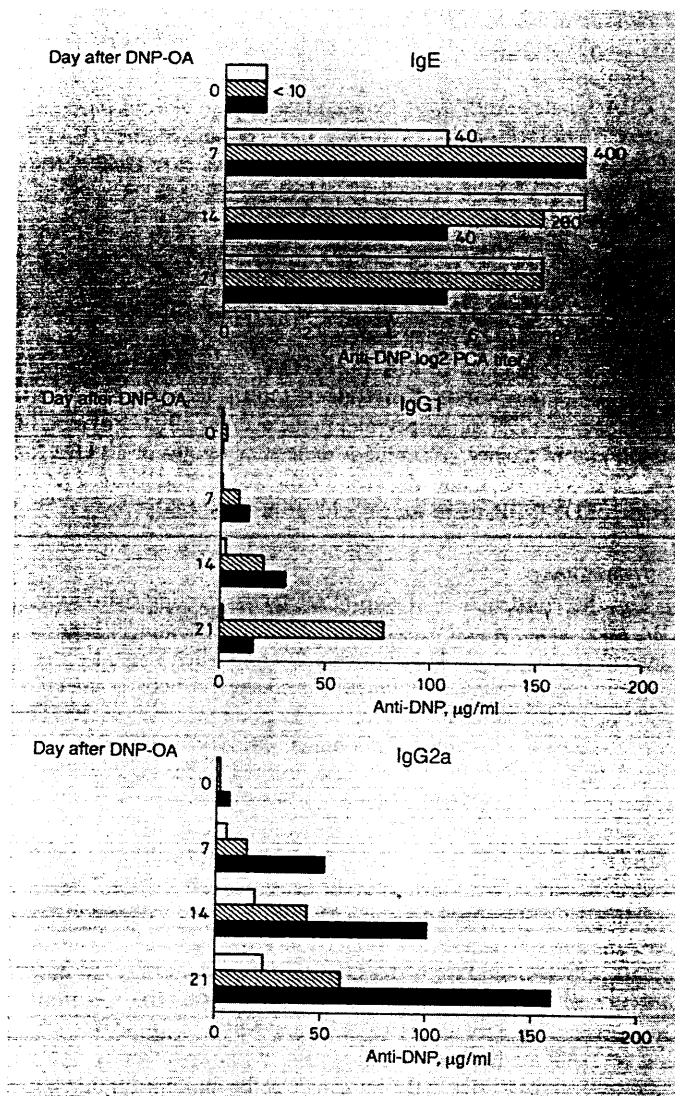


Fig. 1. Effect of pretreatment with DNP-BP on the anti-DNP antibody response of (BALB/cx₃BA)F1 mice. Groups of 5 mice were pretreated with 10^9 (50 μ g) DNP-BP (■), 50 μ g TNP-KLH (▨), or saline (□) 16, 14 and 12 days before immunization with 2 μ g DNP-OA in alum. All mice were bled on days 0, 7, 14 and 21 relative to the day of immunization with DNP-OA, and anti-DNP IgE (log₂ PCA titers), IgG1 and IgG2a (μ g/ml) antibodies were determined on pooled sera from all mice, in each group. Numerical values in the first panel indicate reciprocals of actual PCA titers.

Hybridoma Implantation

Five days before immunization with DNP-OA in alum, both TNP-KLH-pretreated and normal BCF1 mice were injected i.p. with 5×10^5 or 10^6 live hybridoma cells, freshly collected from ascites of pristane-primed CBA or BCF1 mice.

Passive Transfer of Monoclonal Antibodies

Ascites containing anti-DNP Mab were clarified by centrifugation at 10,000 g for 15 min, and the anti-DNP antibody levels were determined by an enzyme immunoassay (EIA). The ascites were then injected i.p. in various amounts to CBA mice, 24 h before immunization with 2 μ g DNP-OA in alum.

Antibody Determinations

IgE antibodies were measured by rat passive cutaneous anaphylaxis (PCA) as previously described [7]. Fourfold or greater differences in PCA titers between serum samples were considered statistically significant. IgG1 and IgG2a antibodies were determined by enzyme immunoassay (EIA), as previously described [6]. Isotype distribution was determined by developing with affinity purified goat anti-mouse IgG1 or IgG2a (Southern Biotechnology Associates, Birmingham AL), followed by biotinylated mouse anti-goat IgG (Jackson ImmunoResearch/Biocan Scientific, Mississauga, Ont.), and alkaline-phosphatase-labelled streptavidin. Anti-DNP IgG1 and IgG2a antibodies were expressed in μ g/ml of undiluted sample, following extrapolation from standard curves constructed with affinity-purified IgG1 (AC2D12) or IgG2a (B4D4) Mab. The antibodies were purified from DNP-BSA coupled to Sepharose 4B (Pharmacia Canada Inc., Baie d'Urfe, P.Q.), by elution with 0.1 M DNP-glycine (Sigma Chemical Co., St. Louis, Mo.), in 0.2 M Tris-HCl buffer pH 8.6 [12].

Statistical Analysis

When appropriate, PCA titers and IgG antibody levels were expressed as geometric means of replicate sera, after logarithmic transformation of the data, and were analyzed by a single factor analysis of variance (one-way Anova), with multiple comparisons of the means [13].

Results

Effect of Pretreatment with DNP-BP on the Anti-DNP Antibody Response of (BALB/cx₃BA)F1 Mice

Since the hybridomas intended for the implantation experiments were constructed by fusion of a BALB/c (H-2^d) myeloma (SP2/0) with CBA (H-2^k)-derived spleen cells, we bred BALB/cx₃BA F1 (BCF1) mice so as to provide the implanted hybridomas with a permissive host. In order to determine whether or not the BCF1 mice responded to pretreatment with DNP-BP or TNP-KLH, in a manner similar to that reported previously for the CBA strain [6], groups of 5 BCF1 males were pretreated with 10^9 (50 μ g) DNP-BP, 50 μ g TNP-KLH or saline, 16, 14 and 12 days before immunization with 2 μ g DNP-OA in alum. The mice were bled on days 0, 7, 14 and 21 after immunization with DNP-OA in alum, and the serum pools on each day were used to determine the amount of IgE, IgG1 and IgG2a anti-DNP antibodies. The results of a representative experiment are shown in figure 1. On day 0, there were no detectable anti-DNP IgE antibodies in either group (PCA titers of <1:10 were arbitrarily assigned a titer of 1:2). On

day 7, there was an accelerated primary response (PCA titer of 1:400) in both pretreatment groups, compared to the saline-injected controls (PCA titer 1:40). On day 14, while the IgE levels of the TNP-KLH-pretreated group remained high (PCA titer of 1:200), the IgE levels of the DNP-BP-pretreated group declined 10 fold relative to the day 7 levels (PCA titer of 1:40). The saline-injected controls, exhibited the expected primary anti-DNP IgE response, with the day 14 peak PCA titer of 1:400. At the time of DNP-OA immunization (day 0), the anti-DNP IgG1 antibodies predominated in the TNP-KLH-pretreated group, while the anti-DNP IgG2a antibodies predominated in the DNP-BP-pretreated group. After immunization with DNP-OA in alum, the groups pretreated with TNP-KLH and DNP-BP exhibited almost equal levels of IgG1 on either day 7 or 14, and a selective sharp increase in the TNP-KLH-pretreated group, on day 21. In the group pretreated with DNP-BP, IgG2a anti-DNP antibodies predominated at all times tested. The same quantitative relationship between IgE, IgG1 and IgG2a were observed in 3 separate experiments in which CBA, BALB/c or BCF1 mice were used (results not shown), indicating that similar immunomodulatory effects operate in these strains after pretreatment with DNP-BP and TNP-KLH respectively.

Effect of Passive Transfer of Anti-DNP IgG1 and IgG2a Monoclonal Antibodies on the Primary Anti-DNP IgE Response

To determine whether the hybridoma-derived anti-DNP monoclonal antibodies had any effect on the development of the IgE response, groups of 5 CBA/J mice were injected i.p. with amounts ranging from 0 to 300 μ g of Mab AC2D12 (IgG1) or B4D4 (IgG2a), followed 24 h later by a primary immunization with 2 μ g DNP-OA in alum. The results in figure 2 clearly show that as little as 10 μ g anti-DNP IgG1 Mab suppressed the primary anti-DNP IgE response. On the other hand, the passive transfer of between 30 and 300 μ g anti-DNP IgG2a antibodies was without any detectable suppressive effect.

Effect of Anti-DNP Hybridoma Implantation on the Anti-DNP Antibody Response of the Adoptive Host

In preliminary trials we determined that the i.p. transfer of 5×10^5 to 10^6 AC2D12 or B4D4 hybridomas, into BCF1 mice resulted in the secretion of sufficient amounts of anti-DNP antibodies to allow the detection in serum of 2-100 μ g/ml anti-DNP antibodies 4-5 days after hybridoma transfer. To determine whether the anti-DNP antibodies produced by the implanted hybridomas had any ef-

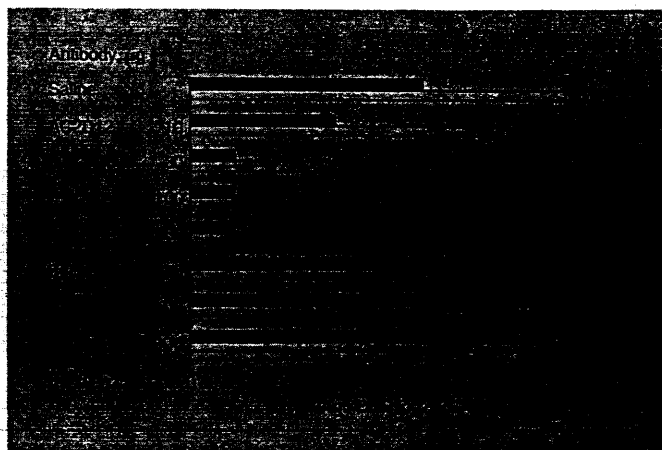


Fig. 2. Effect of passive transfer of anti-DNP monoclonal antibodies on the anti-DNP IgE antibody response. CBA mice were injected with increasing amounts of anti-DNP monoclonal antibody AC2D12 (IgG1), B4D4 (IgG2a), or saline. All mice were immunized 24 h later with 2 μ g DNP-OA in alum. Day 7 (■) and day 14 (□) anti-DNP IgE levels are indicated as log 2 PCA titers. Numerical values in the graph indicate reciprocals of actual PCA titers. The tests were done on pooled sera from all mice, in each group.

fect on the development of a primary anti-DNP IgE response, groups of 6 BCF1 mice were injected with either 10^6 live AC2D12 (IgG1) or B4D4 (IgG2a) anti-DNP-secreting hybridoma cells. A third group was injected with saline. Mice in all groups were immunized with 2 μ g DNP-OA in alum, on day 0 (5 days after hybridoma transfer). The results in Figure 3 show that recipients of B4D4 cells (IgG2a), and the saline control exhibited a normal day 7 IgE response (PCA titer of 1:40), while the recipients of AC2D12 cells (IgG1) had a PCA titer of only 1:10. The low day 7 PCA titer in the recipients of AC2D12 cells coincided with increased day 0 and day 7 anti-DNP IgG1 antibodies of 50 and 4,800 μ g/ml, respectively. These levels were clearly attributable to the antibodies secreted by the hybridoma since both control and recipients of B4D4 (IgG2a) hybridoma had undetectable levels of anti-DNP IgG1 on day 0 (5 days after hybridoma transfer), and only 6 and 8 μ g/ml on day 7, for the saline and B4D4-injected groups, respectively.

Conversely, the recipients of B4D4 cells (IgG2a) had considerable amounts of anti-DNP IgG2a antibodies (20 μ g/ml on day 0, and 1,200 μ g/ml on day 7 after immunization with DNP-OA in alum), but displayed no decrease in anti-DNP IgE levels. Day 14 antibody levels are not available in the hybridoma recipients, since by that time (19 days after hybridoma implantation), all mice had died

Table 1. Effect of anti-DNP hybridoma parking on the IgE response of mice after pretreatment with TNP-KLH

Pretreatment ^a	Hybridoma ^b	IgE ^c		IgG1 ^d			IgG2a ^d		
		7 ^e	14	0	7	14	0	7	14
Saline	none	80	460	<1	3	158	<1	2.4	32
TNP-KLH	none	400	303	4.5	80	225	8	55	140
TNP-KLH	AC2D12 ^f	400	290	22	115	<i>1,540</i>	9	30	120
TNP-KLH	B4D4 ^g	400	190	8	48	340	38	180	<i>1,300</i>
TNP-KLH	AH7 ^h	400	160	10	63	140	9	34	30

^a Groups of 6 (BALB/cx3BA) F1 mice were injected with saline or with 50 µg TNP-KLH i.p., 16, 14, and 12 days before immunization with 2 µg DNP-OA in alum.

^b 5×10^5 live hybridoma cells from freshly collected ascites were injected i.p. 5 days before immunization with 2 µg DNP-OA in alum.

^c Reciprocals of PCA titers. Day 14 titers are geometric means of 4–6 individual sera.

^d Anti-DNP IgG1 or IgG2a antibodies are expressed in µg/ml. Day 14 values are geometric means of 4–6 individual sera.

^e Days after immunization with 2 µg DNP-OA in alum.

^f Anti-DNP IgG1-secreting hybridoma.

^g Anti-DNP IgG2a-secreting hybridoma.

^h Anti-NIP IgG1/IgG2a-secreting hybridoma.

Italicized values are significantly higher than all the other groups ($p < 0.05$ – < 0.001).

from the rapidly expanding tumors. The day 14 serum pool of the saline control had a PCA titer of 1:400, 158 µg/ml IgG1 and 32 µg/ml IgG2a anti-DNP antibodies (not shown). Taken collectively, these results indicate that the implantation of either IgG1 or IgG2a hybridomas determined the appearance of considerable amounts of anti-DNP antibodies in the serum, but only the recipients of IgG1-secreting hybridomas exhibited a suppressed primary anti-DNP IgE response.

Effect of Hybridoma Transfer in Mice Pretreated with TNP-KLH

The results obtained so far indicated that IgG1, and not IgG2a anti-DNP antibodies suppressed primary anti-DNP IgE response. We showed previously, however, that in mice pretreated with DNP-BP, the sharp decline in IgE anti-DNP antibodies coincided with the dominance of the anti-DNP IgG2a isotype [6]. On the other hand, TNP-KLH-pretreated mice exhibited a predominantly IgG1 response but no IgE decline. We considered the possibility that mice pretreated with TNP-KLH might be induced to mimic the pattern of IgE response observed in DNP-BP-pretreated mice provided such mice could either actively or adoptively produce, between the time of TNP-KLH administration and the immunization with DNP-OA in alum, anti-DNP IgG2a antibodies in amounts equal to, or greater than mice pretreated with DNP-BP. Thus, groups of 6 BCF1 mice were pretreated with 50 µg TNP-KLH on

days –16, –14 and –12, and 5×10^5 live hybridoma cells secreting either IgG1 (AC2D12) or IgG2a (B4D4) anti-DNP antibodies were implanted i.p. on day –5. One group was pretreated with TNP-KLH and received an irrelevant hybridoma (AH7) producing anti-NIP IgG1 and IgG2a antibodies, one group was pretreated with TNP-KLH without hybridoma transfer, and one group with saline. All mice were immunized with 2 µg DNP-OA in alum on day 0. The anti-DNP IgE, IgG1 and IgG2a antibody levels on days 0, 7 and 14 are presented in table 1. It can be seen that the recipients of anti-DNP IgG2a hybridomas had 38 µg/ml anti-DNP IgG2a on day 0. Nevertheless, the day 7 anti-DNP PCA titers were no different from those of mice pretreated with TNP-KLH alone, or of those pretreated and implanted with anti-NIP hybridomas (AH7) which had on day 0, 8 and 9 µg/ml anti-DNP IgG2a, respectively. A similar pattern can be seen in mice pretreated with TNP-KLH, and implanted with anti-DNP IgG1-secreting hybridoma cells (AC2D12). There was no decrease in anti-DNP IgE levels on day 7, despite increased anti-DNP IgG1 on day 0 (22 µg/ml) compared to the TNP-KLH-pretreated controls (4.5 µg/ml). On day 14, even in the presence of extremely large amounts of anti-DNP IgG1 (1,540 µg/ml) or IgG2a (1,300 µg/ml), the differences in PCA titers between groups were not statistically significant. Furthermore, there was neither a direct nor an inverse correlation between anti-DNP IgG1 or IgG2a antibody levels on one hand, and PCA titers on the other, when sera from individ-

ual mice were analyzed separately (not shown). These results clearly indicate that the presence in increased amounts, of anti-DNP antibodies of either IgG1 or IgG2a isotypes do not interfere with the production of anti-DNP IgE antibodies if the mice have been pretreated with TNP-KLH, prior to implantation and immunization with DNP-OA in alum.

Discussion

We have shown in a previous report [6], and confirmed here that mice pretreated with DNP-coupled *B. pertussis* vaccine and then immunized with DNP-OA in alum exhibit an accelerated but short-lived primary anti-DNP IgE response followed by a sharp decrease a few days later (fig. 1). We have established also that this decrease was hapten-specific inasmuch as only the anti-DNP and not the anti-carrier response was affected [6]. It is well established that antibody transferred passively [14, 15] or secreted endogenously by myeloma/hybridoma implants into an appropriate host [16, 17] exert powerful regulatory influences on both B and T cell functions. Since IgE antibody production in both mice [18] and rats [19] are known to be extremely susceptible to antibody-mediated suppression, it was reasonable to explore the possibility that the decrease in anti-DNP IgE levels occurring after DNP-BP pretreatment was – at least in part – a consequence of the immunoregulatory effect of anti-DNP IgG2a antibody. The results presented here show that the decrease in anti-DNP IgE after pretreatment with DNP-BP was not attributed to an IgG2a-mediated effect. This conclusion is supported by the finding that neither the passive transfer of up to 300 μ g anti-DNP IgG2a Mab (hybridoma B4D4) nor its endogenous production after hybridoma implantation could mimic the IgE decrease observed after DNP-BP pretreatment, despite the fact that the amounts of anti-DNP IgG2a passively transferred (fig. 2) or present in the serum of the hybridoma recipients at the time of immunization with DNP-OA in alum (fig. 3b) were equal to, or higher than the amounts detected on the same day in the sera of mice pretreated with DNP-BP (fig. 1). In sharp contrast to the apparent lack of effect by IgG2a, the anti-DNP IgG1 Mab determined a profound suppression of the primary anti-DNP IgE response. The recipients of the anti-DNP IgG1 had the day 7 PCA titer 4 times lower than those of the normal (saline) controls, and of the recipients of IgG2a (B4D4) hybridoma (fig. 3a). It should be noted that at the time of immunization with DNP-OA in alum, the serum pool of mice implanted with hybridoma

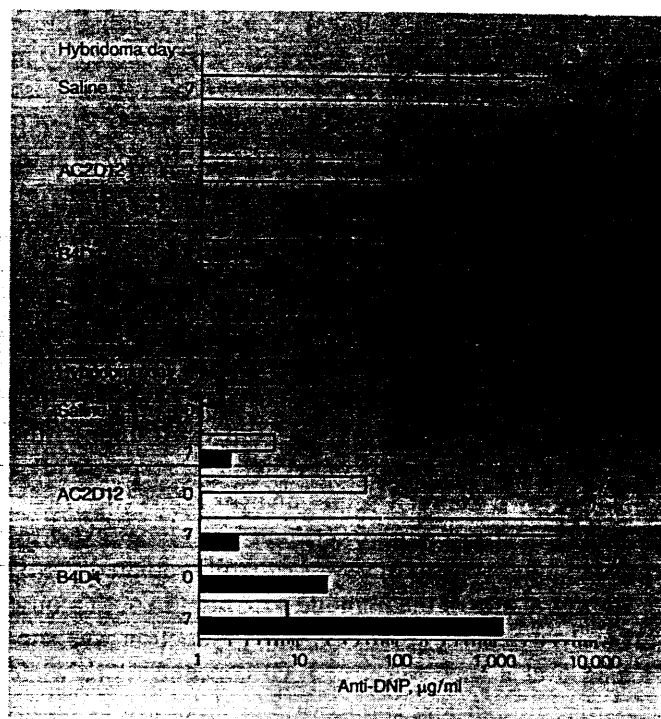


Fig. 3. Effect of hybridoma implantation on the primary anti-DNP antibody response. Groups of 6 BCF1 mice were injected with 10^6 AC2D12 (IgG1), B4D4 (IgG2a) or with saline, i.p., 5 days before immunization with 2 μ g DNP-OA in alum. Anti-DNP IgE antibodies presented as log₂ PCA titers are in (a). Numerical values in (a) indicate reciprocals of actual PCA titers. Anti-DNP IgG1 ($\square$) and IgG2a ($\blacksquare$) expressed in μ g/ml are in (b). The tests were done on pooled sera from all mice, in each group.

AC2D12 had 37 μ g/ml anti-DNP IgG1 antibody. This is equivalent to the amounts exhibiting strong suppressive effects after passive Mab (fig. 2). It has been repeatedly demonstrated that an immune response once induced is more refractory to antibody-mediated suppression than if passive transfer occurs prior to, or shortly after active immunization [18, 20, 21]. The results presented in table 1 are consistent with those observations. Neither IgG1 nor IgG2a anti-DNP Mab interfered with the accelerated primary IgE response. All groups pretreated with TNP-KLH and immunized with DNP-OA in alum had day 7 anti-DNP PCA titers of 1:400, regardless of whether hybridomas were implanted or not. It should be noted that the recipients of hybridoma AC2D12 (IgG1) had 22 μ g/ml anti-DNP IgG1 on the day of immunization with DNP-OA in alum. This value is well within the range of IgG1 antibody which effectively suppressed the primary IgE response in non-pretreated mice (fig. 2, 3). The day 14 PCA

titers of all hybridoma recipient groups – including those of an irrelevant specificity (anti-NIP) – were decreased in comparison to the TNP-KLH pretreated control, and also in comparison to the corresponding day 7 titers. The differences were statistically not significant. In addition to, and independent of antibody-specific suppression, implanted plasma cell tumors have been known to interfere non-specifically with antibody production of the host through a mechanism involving the production of soluble mediators by the implanted tumour and by the host macrophages [22]. In the present investigation, such an inhibitory effect was not yet detectable on day 7 (12 days after hybridoma implantation) and did not contribute to the suppression of the primary anti-DNP IgE response after implantation of the IgG1-secreting hybridoma (fig. 3).

Many investigators reported that Ig isotypes exert selective regulatory properties on both cell-mediated (CMI) and humoral arcs of the immune response. In both mouse [23] and guinea pig [24], antibody production was suppressed over a wide range of IgG1 concentrations while IgG2 was only partially suppressive in high concentrations and enhancing in low concentrations. The mouse IgG2 isotype was not identified. The present results are, to our knowledge, the first to show a clear dissociation between the ability of either passively transferred or endogenously produced monoclonal anti-hapten antibodies of distinct IgG isotypes to suppress the development of a primary anti-hapten IgE response. It appears, therefore, that the decrease in anti-DNP IgE levels after pretreatment with DNP-BP should not be attributed to the increase in anti-DNP IgG2a.

Immunization with certain antigens is known to lead to commitment of CD4⁺ T lymphocytes to either predominantly T_H-1 or T_H-2 subpopulations which secrete IFN- γ and IL-4, respectively [2, 4]. These T cell subpopulations exert mutually inhibitory effects through their respective lymphokines. It has been recently reported that IgG1 and IgG2a anti-TNP Mab exerted regulatory effects on CMI functions. IgG1 enhanced T_H-1-dependent functions such as delayed hypersensitivity, while IgG2a and IgG2b inhibited this function through the generation of CD8⁺ T suppressor cells [25]. It was suggested that the effects of T_H-1 and T_H-2 lymphocytes on the immune response could be selectively reversed by different IgG isotypes, a process which may take place later in the immune response [26]. The present experiments do not substantiate nor do they disprove such a mechanism.

Studies from many laboratories have established that both animal and human B and T lymphocytes express Fc receptors (FcR) for each of the Ig heavy chain classes. It

has been repeatedly suggested that these FcR constitute a key component in immune regulation [27]. For IgG, three different types of FcR have been identified, of which two – Fc γ RII and Fc γ RIII – are present on lymphocytes. There is experimental evidence indicating that Fc γ RII is instrumental in IgG antibody-mediated suppression of B cell function [27]. It is conceivable that the selective suppression of the primary IgE response by IgG1 antibody, but not by IgG2a operates through differential binding of these two isotypes to distinct Fc γ receptors, thus delivering distinct signals to B cells. These signals could be delivered directly to the target B cell, or indirectly through different regulatory T cell subsets bearing distinct Fc receptors on their surface.

Acknowledgements

The authors wish to thank Dr. R.G. Lynch and M. Sandor for reviewing the manuscript, and Miss Anita Evans for secretarial assistance in the preparation of the manuscript.

References

- Ishizaka K: Regulation of IgE synthesis. *Ann Rev Immunol* 1984;2:159-182.
- Mosmann TR, Coffman RL: Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Adv Immunol* 1989;46:111-147.
- Finkelman FD, Holmes J, Katona IM, Urban JF Jr, Beckman MP, Park LS, Schooley KA, Coffman RL, Mosmann TR, Paul WE: Lymphokine control of *in vivo* immunoglobulin isotype selection. *Ann Rev Immunol* 1990;8:303-333.
- Romagnani S: Human TH-1 and TH-2 subsets: Doubt no more. *Immunol Today* 1991;12:256-257.
- Iwata M, Huff T, Uede T, Munoz JJ, Ishizaka K: Modulation of the biologic activities of IgE-binding factor. II. Physicochemical properties and cell sources of glycosylation-enhancing factor. *J Immunol* 1983;130:1802-1808.
- Hagen M, Esani NE, Strejan GH: Role of interferon-gamma in the modulation of the IgE response by 2,4-dinitrophenyl-*Bordetella pertussis* vaccine in the mouse. *Eur J Immunol* 1989;19:441-446.
- Colby WD, Strejan GH: Immunological tolerance of the mouse IgE system: dissociation between T cell tolerance and suppressor cell activity. *Eur J Immunol* 1980;10:602-608.
- Weinberger JZ, Greene MI, Benacerraf B, Dorf ME: Hapten-specific T cell responses to 4-hydroxy-3-nitrophenylacetyl. I. Genetic control of delayed-type hypersensitivity by V_H and I-A region genes. *J Exp Med* 1979;149:1336-1348.
- Schulman M, Wilde CD, Kohler G: A better cell line for making hybridomas secreting specific antibodies. *Nature* 1978;276:269-272.
- Kohler G, Milstein C: Continuous cultures of fused cells secreting antibodies of predefined specificity. *Nature* 1975;256:495-497.
- Potter M: Immunoglobulin-producing tumors and myeloma proteins of mice. *Physiol Rev* 1972;52:631-719.
- Hagen M, Strejan GH: Antigen leakage from immunosorbents: Implications for the detection of site-directed auto-anti-idiotypic antibodies. *J Immunol Methods* 1987;100:47-57.
- Zar JH: Biostatistical analysis. Prentice Hall, Englewood Cliffs, 1974.
- Uhr JW, Moller G: Regulation of effect of antibody on the immune response. *Adv Immunol* 1968;8:81-127.
- Sinclair NRS: Regulation of the immune response. I. Reduction in ability of specific antibody to inhibit long-lasting IgG immunological priming following removal of the Fc fragment. *J Exp Med* 1969;129:1183-1201.
- Hanley-Hyde JM, Lynch RG: The physiology of B cells as studied with tumor models. *Ann Rev Immunol* 1986;4:621-649.
- Mathur A, Maekawa S, Ovary Z, Lynch RG: Increased T_H cells in BALB/c mice with an IgE-secreting hybridoma. *Mol Immunol* 1986;23:1193-1201.
- Ishizaka K, Okudaira H: Reaginic antibody formation in the mouse. I. Antibody-mediated suppression of reaginic antibody formation. *J Immunol* 1972;109:84-89.
- Tada T, Okumura K: Regulation of homocytotropic antibody formation in the rat. I. Feedback regulation by passively administered antibody. *J Immunol* 1972;106:1002-1011.
- Wigzell H: Antibody synthesis at the cellular level. Antibody-induced suppression of 7S antibody synthesis. *J Exp Med* 1966;124:953-968.
- Axelrad MA: Suppression of memory by passive immunization late in the primary response. *J Exp Med* 1971;133:857-863.
- Berman JE, Zolla-Pazner S: Control of B cell proliferation: arrest of B cells in late G1 underlies immunosuppression induced by plasma cell tumors. *J Immunol* 1987;138:2805-2812.
- Murgita RA, Vas SI: Specific antibody-mediated effect on the immune response. Suppression and augmentation of the primary immune response in mice by different classes of antibodies. *Immunology* 1972;22:319-331.
- Vuagnat P, Neveu T, Voisin GA: Immunodepression by passive antibody, an expression of selective immunodepression. I. Action of guinea pig IgG1 and IgG2 anti-hapten antibodies. *Eur J Immunol* 1973;3:90-95.
- Ptak W, Janeway CA Jr, Flood PM: Immunoregulatory role of Ig isotypes. II. Activation of cells that block induction of contact sensitivity response by antibodies of the IgG2a and IgG2b isotypes. *J Immunol* 1988;141:765-773.
- Street NE, Mosmann TR: Functional diversity of T lymphocytes due to secretion of different cytokine patterns. *FASEB J* 1991;5:171-177.
- Lynch RG, Sandor M: Fc Receptors on T and B Lymphocytes; in Metzger H (ed): Fc Receptors and the Action of Antibodies. Washington, American Society of Microbiology, 1990, pp 305-334.