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Suppression of Antigen-Specific Th2 Cell-Dependent IgM and IgG1 Production Following Norepinephrine Depletion In Vivo

A. P. Kohm* and V. M. Sanders2*‡

The mechanism by which the Th2 cell-dependent Ab response is modulated by the sympathetic neurotransmitter norepinephrine (NE) was investigated. Our model system used the severe combined immunodeficient (scid) mouse that was depleted of NE with 6-hydroxydopamine before reconstitution with a clone of β2-adrenergic receptor (β2AR)neg KLH-specific Th2 cells and resting trinitrophenyl (TNP)-specific β2ARpos B cells enriched from the spleens of unimmunized mice. Following challenge with TNP-keyhole limpet hemocyanin (KLH), Ab production in these mice was hapten-, carrier-, and allotype-specific as well as MHC restricted. Depletion of NE resulted in a 50–75% suppression of the primary anti-TNP IgM response compared with that of NE-intact controls, while the secondary IgM response returned to control levels. In contrast, both the primary and secondary anti-TNP IgG1 responses were suppressed by 85 and 40%, respectively. Using NE-intact mice exposed to either a β2AR- or α2AR-selective antagonist, the effect of NE on the Ab response was shown to be mediated by the β2AR. In addition, administration of a β2AR-selective agonist to NE-depleted mice partially reversed the suppressed Ab response that resulted from NE depletion. Expression of the β2AR on TNP-specific B cells was confirmed by radioligand binding, immunofluorescence, and cAMP analysis. Also, while splenic histology was comparable in NE-intact and NE-depleted mice before Ag exposure, follicle expansion and germinal center formation were suppressed in NE-depleted mice after Ag exposure. Taken together, these results suggest that NE stimulation of the β2AR expressed on B cells is necessary for the maintenance of an optimal primary and secondary Th2 cell-dependent Ab response in vivo. The Journal of Immunology, 1999, 162: 5299–5308.

The immune system has developed numerous strategies for the regulation of lymphocyte development and function. One strategy involves intracellular signaling mechanisms that are induced by either cytokines or cell-associated receptor-ligand interactions (reviewed in Ref. 1). Another strategy involves a bidirectional communication pathway between the CNS and the immune system. Evidence for the ability of the immune system to signal the CNS includes the presence of cytokine receptors within the CNS (reviewed in Ref. 2) and on peripheral sympathetic nerves and ganglia (3–6). Likewise, evidence for the reciprocal component of this bidirectional pathway includes the presence of sympathetic nerve fibers in both primary and secondary lymphoid organs and the presence of adrenergic receptors (ARs)3 for the sympathetic neurotransmitter norepinephrine on lymphocytes (reviewed in Ref. 7). Therefore, the existence of a bidirectional communication pathway between the CNS and the immune system suggests a possible role for the CNS in the maintenance of immune homeostasis as well as in the development and progression of immune-related disease states.

Many groups have described the innervation of both primary and secondary lymphoid organs by sympathetic nerve fibers. These studies demonstrate rich sympathetic innervation penetrating the parenchyma of lymphoid organs via the vasculature that supplies the periaorterinal lymphoid sheath (PALS), marginal zone, and marginal sinus (8). In addition, electron microscopic studies reveal that sympathetic nerve terminals are in direct apposition to T cells and interdigitating dendritic cells (8), with this junction being ~6 nm wide compared with a typical CNS synapse, which is ~20 nm wide. Following exposure to Ag (9, 10), LPS (11), or IL-1 (11) in vivo, the sympathetic neurotransmitter norepinephrine (NE) is released from sympathetic nerve terminals and is bound by the β-adrenergic receptor (βAR) expressed on resident lymphocytes, which induces an increase in the intracellular concentration of cAMP (7).

A variety of murine immune cells express the β2AR, including macrophages, NK cells, B cells, and T cells (reviewed in Ref. 7). Recent data show that the β2AR is differentially expressed on both resting and anti-CD3-activated murine CD4+ effector T cell clones, with detectable expression on Th1 cells, but not on Th2 cells, as determined at the protein level by radioligand binding and immunofluorescence analysis (12, 13), at the mRNA level by RT-PCR (A. P. Kohm et al., manuscript in preparation), and at the functional level by cAMP accumulation following receptor stimulation with a β2AR-selective agonist (12). Furthermore, the functional relevance of differential β2AR expression on Th1 and Th2 cell clones was demonstrated by the finding that β2ARneg Th1 clones pre-exposed to a β2AR-specific agonist before interaction with nonexposed Ag-presenting B cells in vitro produced less IFN-γ than nonexposed Th1 cell controls and resulted in reduced IgG2a production by the B cells (12). As expected, β2ARneg Th2 clones preexposed to a β2AR-specific agonist before interaction with nonexposed B cells produced the same amount of IL-4 as nonexposed Th2 clones and resulted in no change in IgG1 production by the Ag-presenting B cells. In contrast, preliminary findings in our laboratory suggest that stimulation of the β2AR on B

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3 Abbreviations used in this paper: AR, adrenergic receptor; PALS, periaorterinal lymphoid sheath; NE, norepinephrine; 6-OHDA, 6-hydroxydopamine; KLH, keyhole limpet hemocyanin; TNP, trinitrophenyl; FLU, fluorescein; PNA, peanut agglutinin; IPIN, iodopindolol.

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cells during Ag processing, but before interaction with β2AR
Th2 cell clones, results in an enhancement of IgG1, but not IL-4,
production (D. Kasprowicz et al., manuscript in preparation). Thus, the lack of β2AR expression on clones of Th2 effector cells provides a unique opportunity to study the possible role played by this receptor that is expressed on B cells alone in the NE-induced modulation of the Th2 cell-dependent Ab response both in vitro and in vivo.

In previous studies to determine the role of NE in modulating the Th cell-dependent Ab response in vivo, peripheral sympathetic nerve terminals containing NE were reversibly destroyed in animals by chemical sympathectomy with the neurotoxin 6-hydroxydopamine (6-OHDA) before exposure to Ag. Results from experimental model systems using 6-OHDA-treated animals have been conflicting, showing enhancement (9, 14, 15), suppression (16–18), or no change (19) in the level of Ab production and cell proliferation. One possible source of these conflicting results may be the low frequency of Ag-specific lymphocytes in previous an-
proliferation. One possible source of these conflicting results may be the low frequency of Ag-specific lymphocytes in previous an-

Materials and Methods

Animals

Six-week-old female C.B.-17/ICR (H-2d, IgG-Cγ), C.B.-17/ICR scid (H-2d, IgG-Cγ), BALB/c (H-2d, IgG-Cγ), and B6C3F1 (H-2d) mice were obtained from Harlan Sprague-Dawley (Indianapolis, IN). All mice were provided autoclaved pellets and water ad libitum. The scid mice received tetracycline HCl (2 mg/ml; Pfizer, New York, NY) in their drinking water 3 days/wk. Mice were permitted 2 wk to acclimate to their environment before being manipulated and were used at 8 wk of age in all experiments. The scid mice were housed under a 12-h light, 12-h dark cycle in microiso-
later cages contained within a laminar flow system thus maintaining a pathogen-free environment, and all experimental manipulations occurred −4 h into the light cycle.

Reagents and Abs

2,4,6-Trinitrobenzenesulfonic acid was purchased from Fluka (Ronkonkoma, NY), OVA, trinitrophenyl (TNP), and fluorescein (FLU) were purchased from Sigma (St. Louis, MO), and KLH was obtained from Calbiochem (La Jolla, CA). TNP-KLH, TNP-FLU, or FLU-KLH were prepared at a haptenation ratio of 17–24 TNP or FLU molecules/KLH or OVA carrier molecule. Terbutaline, nadolol, phentolamine, and metapro-
teranol were purchased from Sigma. All pharmacologic agents were dissolved in sterile PBS immediately before administration in vivo. Abs used in the ELISA were purchased from Pharmingen (San Diego, CA). A rabbit polyclonal IgG directed against an epitope corresponding to aa 399–418 mapping to the carboxyl terminal of the β2AR of mouse origin and control peptide sequences (aa 399–418) were purchased from Santa Cruz Bio-
technology (Santa Cruz, CA).

Cell transfer and immunization

Two days following the last 6-OHDA injection, all animals received both BAC-3.2 Th2 cells and TNP-specific B cells. Each cell type was prepared for adoptive transfer at 2 × 10^6 cells in 50 μl of PBS. T and B cell dilutions were prepared separately and were combined only at the time of injection. Cells were injected i.v. into the lateral tail vein in a total volume of 100 μl of PBS. One week after cell reconstitution, sympathectomized animals received primary immunizations i.p. with 100 μg of TNP-OVA, TNP-KLH, or FLU-KLH, delivered in the adjuvant TiterMax Gold (CytRx, Norcross,
GA). In some experiments, mice received an i.p. injection of a β2AR-selective agonist (5 mg/kg terbutaline or metaproterenol; Sigma), a βAR-selective antagonist (5 mg/kg nadolol; Sigma), or saline at the time of Ag injection.

Detection of secreted Ag-specific and allotype-specific anti-TNP IgM and IgG1

Briefly, 96-well, round-bottom Immulon plates (Dynatech, Chantilly, VA) were coated with 100 μg/ml of TNP-OVA overnight at 4°C, washed with PBS, and blocked with PBS/1% BSA for 1 h at 37°C. Ab-containing serum, diluted between 1/100 to 1/1000, was added to each well. Plates were incubated for 2 h at 37°C in a humidified atmosphere. Wells were washed with PBS/0.05% Tween-20. For Ag-specific assays, a 1/2000 dilution of alkaline phosphatase-conjugated goat anti-mouse IgM or IgG1 Ab (Southern Biotechnology Associates, Birmingham, AL) was added to each well for 1 h at 37°C, and nonspecific binding was blocked with PBS/1% BSA for 1 h at 37°C. The microtiter tubes were incubated for 1 h at 37°C and then filtered onto glass fiber filters using a Cell Harvester (Brandel, Gaithersburg, MD). After five washes using a cold buffer (0.45 M Tris-HCl and 0.170 M MgCl2·6H2O; pH 7.2) the samples were counted in a Beckman 5500 B gamma counter (Beckman, Palo Alto, CA) with a calculated accuracy of 87%. Both total and nonspecific binding tubes were run for each time point. Results are reported in picomols of IPIN bound. The nonspecific binding was determined in the tubes containing (–)propranolol at a final concentration of 1 μM. Specific binding of radioligand was determined by subtracting nonspecific binding from the counts occurring in the total binding tubes for each concentration of radioligand used. Binding curve data were analyzed using the computer program LIGAND (24).

cAMP measurement

Assays were performed using B cells at a density of 1 × 10^6 cells/ml in serum-free HBSS containing 10 mM HEPES and 1 mM 3-isobutyl-1-methylxanthine (Sigma) to inhibit phosphodiesterase activity. After the addition of terbutaline and incubation at 37°C for 30 min, the reaction was terminated by the addition of 3 parts cold ethanol. The soluble fraction was collected by centrifugation and lyophilized. cAMP was measured using a nonisotopic immunoassay system for cAMP (Life Technologies). Results are expressed as picomols of cAMP per 10^6 cells.

Immunofluorescence staining

B cells were resuspended at 5 × 10^6 cells/ml and were fixed for 20 min at room temperature in 2% paraformaldehyde. Cells were stained using a modification (25) of a previously described procedure (26). All staining Abs and detection reagents were purchased from Santa Cruz Biotechnology. All reagents and washes were performed in the presence of 1% BSA and 0.05% saponin (Sigma), and incubations were conducted at room temperature. Cells were first incubated for 10 min in PBS/BSA/saponin and then stained for 30 min with a polyclonal rabbit anti-mouse β2AR Ab (1 μg/ml final concentration) in the absence or the presence of a mouse β2AR-specific peptide sequence (10 μg/ml final concentration) that corresponds to the epitope of the β2AR recognized by the anti-mouse β2AR Ab. Cells were washed and incubated for 30 min in the tubes containing a biotinylated secondary Ab in PBS/BSA/saponin followed by FITC-streptavidin. Cells were washed twice with PBS/BSA/saponin and twice with PBS/BSA without saponin to allow membrane closure. Samples were analyzed on a FACStar Plus flow cytometer (Becton Dickinson, San Jose, CA) gated on all viable cells. Data analysis was performed daily using Rainbow Calibration Particles (Sherotech, Libertyville, IL). Results were analyzed using LYSIS II software (Becton Dickinson).

Statistics

Concentration-response data were first analyzed by a one-way ANOVA to determine whether an overall statistically significant change existed previous to using two-tailed unpaired Student’s t test. Statistically significant differences are reported at p < 0.05.

Results

Immun specificity of the Ab response in reconstituted scid mice

To determine the Ag specificity of the Ab response in scid mice reconstituted with a clone of KLH-specific Th2 cells and TNP-specific B cells, mice were immunized with TNP-KLH, TNP-OVA, FLU-KLH, or adjuvant alone and evaluated for their ability to produce anti-TNP IgM. As shown in Fig. 1, a significant level of anti-TNP IgM was detected in reconstituted mice immunized with TNP-KLH. In contrast, mice receiving either TNP-OVA or...
Th2 cells (BAC 3.2) and TNP-specific B cells. Reconstituted mice were injected i.p. with 100 μg of TNP-KLH, TNP-OVA, FLU-KLH, or adjuvant only. Serum anti-TNP IgM was analyzed by ELISA 2 wk later. In addition, some mice were reconstituted with B cells of a mismatched haplotype. Data are presented as the mean micrograms per milliliter of anti-TNP IgM ± SE from five mice per group. Significant differences from adjuvant-only mice that were reconstituted with B cells of the I-A^d haplotype are indicated by an asterisk when p < 0.05.

FLU-KLH produced levels of anti-TNP IgM that were considerably lower than those in TNP-KLH-injected mice, but were comparable to those in adjuvant-only injected controls. Thus, these data suggest that hapten-carrier specificity exists for the observed anti-TNP response and that TNP-specific B cells bind TNP-KLH, process it, and present the KLH peptide to the KLH-specific Th2 cell clone.

Next, to determine whether an MHC-restricted cognate interaction occurred between the Th2 cell clone and the B cell to allow for the production of TNP-specific Ab, scid mice were reconstituted with a KLH-specific Th2 cell clone that was H-2^d restricted and with TNP-specific B cells enriched from the spleens of unimmunized mice of either a matching haplotype (BALB/c;H-2^d) or a nonmatching haplotype (B6C3F1; H-2^k). The mice were challenged with TNP-KLH, and serum anti-TNP IgM was measured. Mice reconstituted with H-2^d-expressing B cells produced a significant level of serum anti-TNP when challenged with TNP-KLH, while mice reconstituted with H-2^k-expressing B cells produced no detectable level of serum anti-TNP IgM (Fig. 1). Thus, the lack of detectable serum anti-TNP IgM in mice reconstituted with TNP-specific B cells of different haplotypes suggests that an MHC-restricted cognate interaction between the TNP-specific B cell and KLH-specific Th2 cell is necessary for an appreciable level of anti-TNP IgM to be produced.

While the scid mutation is a homozygous (−/−) mutation, it is not absolute. “Leakiness” of the mutation is defined as the appearance of mature T and B cells in the periphery of CB.17 scid mice in an age- and species-dependent manner (27), but this is less common in CB.17/ICR scid mice. To determine whether leakiness occurred in reconstituted scid mice at any time after immunization, serum Ab was checked for allotype specificity. It is known that B cells of BALB/c mice produce the Ab allotype Ig^h^, while B cells of the sibling pairs (+/−) of the scid mouse produce the Ab allotype Ig^h^ (28). As shown in Fig. 2, scid mice (−/−) reconstituted with TNP-specific B cells isolated from BALB/c mice produced IgM and IgG1 only of the allotype Ig^h^. In addition, serum anti-TNP IgM was almost sixfold higher in reconstituted scid mice than in sibling controls, suggesting that the frequency of KLH-specific T cells and TNP-specific B cells was greater in the reconstituted scid mice than in sibling pairs. This result was obtained with all serum samples analyzed regardless of mouse age. Thus, these results suggest that the donor B cells, as opposed to leaky scid B cells, produced the serum anti-TNP Ab in our model system.

Effect of NE depletion on serum IgM and IgG1 levels in scid mice reconstituted with a clone of KLH-specific Th2 cells and TNP-specific B cells

Administration of 6-OHDA to mice induces a long lasting, but reversible, chemical sympathectomy that results in the depletion of peripheral stores of NE (10, 14, 29). A previous study in rats showed that splenic NE levels decrease ~90–95% immediately following 6-OHDA exposure and return to control levels within 56 days post-6-OHDA exposure, as determined by HPLC (29). To determine the effect of chemical sympathectomy on splenic NE levels in our scid model system, 8-wk-old scid mice were exposed to 6-OHDA dissolved in an ascorbate solution for 3 alternating days 2 days before reconstitution with a clone of KLH-specific Th2 cells and TNP-specific B cells. Compared with ascorbate-injected controls, ~95% of the NE stores were depleted in the cellular/supernatant portion of the spleen 2 days following the last 6-OHDA injection, a time point that corresponded to the time of cell reconstitution in the scid model (Fig. 3A). NE remained ~91% depleted in the cellular/supernatant fraction 9 days following the last 6-OHDA injection, a time when primary immunization with TNP-KLH was performed. Approximately 9 wk following chemical sympathectomy (8 wk following primary immunization), a time when secondary immunization was performed, splenic NE levels returned to ~62% of the level in NE-intact control mice, but to 83% of the level in NE-intact mice injected with the vehicle ascorbate. Thus, NE can be depleted almost completely from the spleens of scid mice before cell reconstitution and remain almost completely depleted at the time of cell reconstitution and primary immunization, but NE levels return closer to the levels in ascorbate-injected control mice by the time of secondary immunization.

To determine the effect of NE depletion on the ability of reconstituted scid mice to produce IgM and an Ab isotype characteristic...
of that produced by B cells provided “help” by a Th2 cell, i.e., IgG1, NE-depleted or NE-intact scid mice were reconstituted with Ag-specific Th2 cells and B cells and immunized with TNP-KLH. As shown in Fig. 3B, the primary IgM response in NE-depleted mice was suppressed 50–75% compared with that of NE-intact controls, while the secondary IgG1 response returned to control levels. In contrast, both the primary and secondary IgG1 responses were suppressed by ~85% in NE-depleted mice compared with those in NE-intact mice, but by the second week following the secondary immunization, IgG1 production recovered to ~40% of control levels. However, it may be important to note that while NE depletion suppressed the Ab response, it never completely inhibited the response. IgG3 and IgG2a responses were also detected in the sera of reconstituted scid mice, but their levels were ~200- to 300-fold lower than the levels of IgG1. In addition, both IgA and IgE were undetectable in the serum of reconstituted scid mice. Finally, anti-TNP IgM and IgG1 responses were either <6 μg/ml or undetectable in animals that received adjuvant only injections (data not shown). Thus, these results suggest that NE plays a role in maintaining an optimal level of Th2 cell-dependent IgM and IgG1 production in normal mice.

To determine whether NE depletion influenced successful lymphocyte homing in scid mice following cell reconstitution, spleen sections from either NE-depleted or NE-intact reconstituted scid mice before Ag exposure were stained for Th cells and B cells using Abs directed against the markers CD4 and B220, respectively. One week following i.v. injection, transferred B220+ cells (Fig. 4, A and B) and CD4+ T cells (Fig. 4, C and D) successfully homed to and populated the splenic white pulp of reconstituted scid mice. B220+ cells formed tight organized coronas that were adjacent to the less organized CD4+ T cell containing PALS. In addition, lymphocyte homing and population of both the B cell-containing corona and the T cell-containing PALS were equivalent in both NE-intact (Fig. 4, A and C) and NE-depleted (Fig. 4, B and D) mice, thus suggesting that NE depletion did not affect the ability of the T and B cells in our model system to successfully home to the spleen following i.v. injection into scid mice.

In addition, hematoxylin- and eosin-stained spleen sections were examined before and following Ag exposure. Due to a lack of T and B cells, spleens from nonreconstituted scid mice had limited cellular organization and were devoid of follicles (Fig. 5A) compared with immunocompetent sibling pairs (Fig. 5C). In contrast, spleens from scid mice reconstituted with a clone of Th2 cells and TNP-specific B cells had increased cellular organization into follicles regardless of the NE status of the mice (Fig. 5, B and E), suggesting that successful homing and cellular compartmentalization of the transferred lymphocytes occurred similarly in both NE-intact and NE-depleted mice. Following Ag administration, clonal expansion and germinal center formation were evident in NE-intact mice (Fig. 5C), but were less evident in NE-depleted animals (Fig. 5F). Taken together, these results suggest that NE depletion does not affect the ability of the Th2 cell clones and B cells used in our model to successfully home to the spleen; however, following Ag exposure, less cellular organization into follicles was observed in NE-depleted mice compared with that in NE-intact control mice.

To further investigate the effect of NE depletion on germinal center formation, spleen sections were stained with FITC-labeled PNA 2 wk following secondary Ag exposure. Germinal centers were evident in the NE-intact mice (Fig. 6, A and C), whereas no detectable germinal centers were observed in NE-depleted mice (Fig. 6, B and D). However, it is important to note that PNA+ germinal center B cells were diffusely scattered throughout the spleen in both groups, suggesting that germinal center formation in NE-depleted mice may be delayed. Thus, it appears that Ag-specific Th2 cells and B cells successfully localized to the spleen in both NE-intact and NE-depleted mice, and following Ag exposure, clonal expansion and germinal center formation were significantly
lower in NE-depleted mice compared with those in NE-intact controls.

The AR subtype responsible for mediating the effects of NE on the Ag-specific immune response

All the above data collected from NE-depleted mice compared with NE-intact mice suggested that NE played a role in maintaining an optimal level of Th2-dependent IgM and IgG1 production in normal mice. To determine the AR subtype responsible for mediating this effect of NE on the in vivo immune response, the aAR-selective antagonist phentolamine and the bAR-selective antagonist nadolol were injected into reconstituted NE-intact scid mice at the time of Ag exposure to block specific receptor subtypes that could potentially be stimulated by NE. Mice receiving nadolol had lower serum titers of IgM 2 wk following Ag challenge, whereas animals receiving phentolamine had serum titers equivalent to those of saline-injected controls (Fig. 7). Thus, these results suggest that NE modulates the in vivo Ab response via stimulation of the bAR as opposed to the aAR.

In the present study we used a clone of Th2 cells specific for KLH and a population of murine B cells specific for TNP to reconstitute scid mice. We showed previously that a panel of murine Th2 cell clones do not express a detectable level of the b2AR in either the resting (12) or the activated state (13) compared with either Th1 clones or murine splenic CD4+ T cells (30, 31). To determine whether the b2AR on TNP-specific B cells was of the b2AR-specific subtype, TNP-specific B cells were exposed to an Ab directed against the b2AR-specific peptide sequence of the cytoplasmic N-terminus of the b2AR protein. As shown in Fig. 8A, Scatchard transformation of saturation binding data showed that TNP-specific B cells expressed ~620 binding sites/cell with an affinity (K_d) of 0.1 nM. These values are similar to those reported for unfractionated murine splenic B cells, but higher than those reported for murine Th1 cell clones or splenic CD4+ T cells. To determine whether the b2AR expressed by TNP-specific B cells was of the b2AR-specific subtype, TNP-specific B cells were exposed to an Ab directed against the b2AR-specific peptide sequence of the cytoplasmic N-terminus of the b2AR protein. As shown in Fig. 8B, TNP-specific B cells expressed a detectable level of the N terminus of the b2AR, the specificity of which was shown with blocking peptides specific for the immunizing sequence of the b2AR, but not the b1AR. To determine whether the b2AR on TNP-specific B cells was functional, B cells were examined for their ability to accumulate cAMP intracellularly following exposure to the specific b2AR agonist terbutaline. As shown in Fig. 8C, B cells that were exposed to increasing concentrations of terbutaline showed a concentration-dependent increase in the intracellular cAMP concentration. Taken together, these data suggest that an enriched population of splenic TNP-specific B cells expresses a functional b2AR protein.

To determine whether the suppressed Ab response in the absence of NE was due to a lack of b2AR stimulation on the B cell, reconstituted NE-depleted mice were injected with increasing concentrations of either the b2AR-specific agonist terbutaline or metaproterenol at the time of Ag exposure. Results showed that the

FIGURE 4. Detection of CD4+ and B220+ cells in the spleens of scid mice following i.v. reconstitution. One week following reconstitution, sections of spleen from NE-intact scid mice (A and C) or NE-depleted scid mice (B and D) were stained for either B220+ cells (A and B) or CD4+ T cells (C and D) as indicated by black staining. Central arteries are marked in all panels with an arrow. A and B are X325 magnification, and C and D are X200 magnification. The photomicrographs presented are representative of four mice analyzed per group.

FIGURE 5. Spleen histology of reconstituted scid mice following Ag exposure. Sections of spleen from a nonreconstituted scid mouse (A), an NE-intact reconstituted scid mouse before Ag exposure (B), an NE-intact reconstituted scid mouse 2 wk following Ag exposure (C), a sibling pair mouse (D), an NE-depleted reconstituted scid mouse before Ag exposure (E), and an NE-depleted reconstituted scid mouse 2 wk following Ag exposure (F). Paraffin-embedded spleen sections were stained with hematoxylin and eosin. The photomicrographs presented are representative of six mice analyzed per group.
suppressive effect of NE depletion on Ab production was partially reversed in a dose-dependent manner by either terbutaline or metaproterenol (Fig. 9). In addition, serum anti-TNP IgM was significantly increased in animals receiving chronic injections of metaproterenol each day following NE depletion until the time of Ag exposure. Thus, these results suggested that the Th2 cells and B cells used to reconstitute NE-depleted scid mice remained viable, that the B cells remained responsive to b2AR stimulation, and that the primary consequence of NE depletion may have been to suppress the ability of these cells to function optimally in response to the Th cell-dependent Ag TNP-KLH.

Discussion

In this study we have shown that a population of resting TNP-specific B cells enriched from the spleens of unimmunized mice possess a functional b2AR and that stimulation of this receptor by NE in vivo is necessary to maintain an optimal Th2 cell-dependent IgM and IgG1 response. The ability of NE to maintain optimal Th2 cell-dependent Ab production in NE-intact reconstituted scid mice was suppressed by administration of an antagonist to the bAR, but not by administration of an antagonist to the aAR. In addition, the suppressive effect of NE depletion on Ab production was partially reversed by administration of a b2AR-specific agonist to NE-depleted mice. Although no difference was observed in lymphocyte trafficking or cellular organization within the spleens of either NE-depleted and NE-intact reconstituted scid mice before Ag exposure, the extent of follicle expansion and germinal center formation was suppressed in NE-depleted animals following Ag exposure. Taken together, these data suggest that stimulation of the b2AR on the B cell maintains an optimal level of Th2 cell-dependent Ab production in vivo.

Before Ag exposure, there was no detectable effect of NE depletion on either splenic structure or the ability of lymphocytes to properly home to the spleen in reconstituted scid mice compared with that in NE-intact mice. While it is possible that some of the
cells homed to the peripheral lymph nodes or mucosal lymphoid tissue, it is unlikely, since the T cell clones used in this study are L-selectin negative and are, therefore, unable to cross high endothelial venules to enter the lymph nodes following i.v. reconstitution (32). However, our finding does not support a previous report suggesting that NE depletion in mice prevents lymphocytes from homing properly and that a higher accumulation of adoptively transferred T cells was evident in the spleen and lymph nodes of mice when the T cells were pretreated with a β2AR-selective agonist (33). Thus, the effect of NE depletion on lymphocyte homing remains unclear. Also, the splenic histology of our mice showed that follicular clonal expansion and germinal center formation were less evident following Ag exposure in NE-depleted mice than in NE-intact controls. One possible explanation for this finding is that the lymphocytes present within the spleens of NE-depleted mice may have been in an unresponsive state. However, these cells were able to respond to mitogen stimulation in vitro (data not shown) and appeared to function to some degree following secondary immunization when the level of NE returned to that in controls.

It is possible that the return of IgM production to control levels after secondary immunization was due to a primary response from newly formed B cells migrating from the bone marrow of scid mice following the recovery of NE levels in the spleen. However, this possibility is unlikely, since scid mice rarely generate newly formed B cells (34), and the only Ab allotype produced in the present study was that of the donor cell type. This possibility is also supported by the present findings that serum anti-TNP IgM returned to a control level in NE-depleted scid mice following secondary exposure to Ag and that the suppressive effect of NE depletion on lymphocyte homing remains unclear. Also, the splenic histology of our mice showed that follicular clonal expansion and germinal center formation were less evident following Ag exposure in NE-depleted mice than in NE-intact controls. One possible explanation for this finding is that the lymphocytes present within the spleens of NE-depleted mice may have been in an unresponsive state. However, these cells were able to respond to mitogen stimulation in vitro (data not shown) and appeared to function to some degree following secondary immunization when the level of NE returned to that in controls.
of NE depletion was partially reversed by the β2AR-selective agonist terbutaline. Thus, the production of secondary IgM and IgG1 in our model system may be due to the long term survival of the donor cells or the expansion of this original population during the primary immune response.

In contrast to the present results showing a suppressive effect of NE depletion on Th2 cell-dependent Ab production, one study by Kruszewski et al. (14) reported a strain-specific enhancement of both cytokine and Ab production in NE-depleted C57Bl/6J (Th1-slated strain) and BALB/c (Th2-slated strain) mice. These studies showed that 6-OHDA treatment of mice enhanced primary serum anti-KLH IgM and IgG in KLH-primed C57Bl/6J mice, while NE depletion increased only IgG1 in KLH-primed BALB/c mice. Thus, the effects of NE depletion by 6-OHDA on immune cell function are varied depending on the target organ cells studied, the stimulus used for cell activation, the mouse strain used for study, whether a primary or a secondary response is being measured, and whether the response being measured is generated in vivo or in vitro.

It is also important to note that previous NE depletion studies in vivo used immunocompetent mice with intact mechanisms of precursor development and differentiation to study the role of NE depletion on immune cell function. Thus, since NE and β2AR stimulation have been reported to influence hemopoiesis and modulate the numbers of immune cell progenitors produced in the bone marrow (36), it is possible that NE depletion may remove the ability of this neurotransmitter to influence hemopoiesis in the bone marrow of immunocompetent mice and contribute to the previously discussed conflicting findings. In addition, intracellular cAMP levels have been shown to influence the path of CD4+ T cell differentiation into Th1 and Th2 effector cells (37), suggesting that NE and/or β2AR stimulation may affect the composition of the effector cell population that predominates in NE-depleted immunocompetent mice. Therefore, any conclusions drawn from a study addressing the effects of NE on a resultant immune response in intact animals must also take into consideration the effects of β2AR stimulation on both the developmental and effector stages of the response. We have attempted to address some of these possibilities by establishing a model system in which the mouse possesses a high frequency of Ag-specific T and B cell effector populations that cannot be supplemented by newly formed lymphocytes. Thus, our model system eliminates the possibility that the effect of NE depletion on Ab production is due to an effect on lymphocyte development or differentiation and thereby permits study of the role of NE in modulating a Th2-dependent Ab response to a soluble protein Ag when there is a high frequency of both β2ARmTh2 cells and β2ARmTh2 cells responding to Ag in a hapten-, carrier-, and MHC-restricted manner.

One concern with all in vivo investigations is the contribution of stress and glucocorticoids to the response. While 6-OHDA does not cross the blood-brain barrier in adult mice to enter the CNS, Callahan et al. (38) showed that 6-OHDA administration to mice increased both Fos protein and corticotropin-releasing factor expression on cells residing within the hypothalamus. This increase in Fos protein within the hypothalamus is believed to have resulted from the peripheral nerve terminal damage induced by 6-OHDA, which, by some mechanism, stimulated the hypothalamic-pituitary-adrenal axis to release glucocorticoids from the adrenal cortex. Therefore, a decreased Ab response in vivo following 6-OHDA-induced depletion of NE may be due to the immunosuppressive effects of glucocorticoids and not to a loss of β2AR stimulation by NE. However, another study showed that administration of the glucocorticoid receptor antagonist RU486 in vivo was unable to prevent the effects induced by 6-OHDA (39). Therefore, although 6-OHDA administration may enhance Fos protein and corticotropin-releasing factor expression within the hypothalamus, it appears unlikely that a glucocorticoid effect was responsible for the observed immunosuppressive effects of NE depletion in the present study. However, this possibility will be tested in future studies using our model system.

If stimulation of the β2AR by NE is necessary to maintain optimal Th2 cell-dependent Ab production in vivo, as suggested by the present study and others (12, 13), and if β2AR stimulation leads to an increase in the intracellular concentration of cAMP within the lymphocytes responsible for Th cell-dependent Ab production, then the influence of cAMP accumulation on the ability of lymphocytes to function during a response to Ag needs to be understood. It is possible that the lack of NE stimulation during the primary response to Ag results in a loss of a cAMP-induced effect in the B cell that may remain with the cell until the time of secondary Ag exposure. Such an effect may include the loss of a cAMP-induced effect to either modulate the level of expression of a cell surface molecule on the B cell that is important for optimal B cell function or alter the level of responsiveness of the B cell to cytokines. For example, in vitro stimulation of B cells by either dibutyryl cAMP (40, 41), a β2AR-selective agonist (D. Kasprowicz et al., manuscript in preparation), or NE (D. Kasprowicz et al., manuscript in preparation) enhanced the level of expression of the costimulatory molecule B7-2 on the B cell. Thus, B7-2 expression may be lower on the B cells of mice delected of NE, since these B cells would not receive a cAMP signal via β2AR stimulation to induce optimal B7-2 expression. As a consequence, it is possible that the lack of enhancement of B7-2 expression on the B cell may hinder optimal B cell function, such as Ab production (D. Kasprowicz et al., manuscript in preparation) and optimal germinal center formation (42). Also, the responsiveness of the B cell to IL-4 may be directly affected by cAMP elevation in the B cell, since there is an apparent role for cAMP in enhancing the level of B cell responsiveness to the Th2 cell-derived cytokine IL-4 (43, 44). Since IL-4 stimulation is necessary for up-regulation of MHC class II on B cells (45, 46), B cell proliferation (47, 48), and B cell switch to IgG Ab production (49–52), it is possible that depletion of NE in vivo will remove a signal that is necessary to generate cAMP in a B cell to increase responsiveness to IL-4. Therefore, the possibility exists that the levels of both B7-2 expression and IL-4 responsiveness by the B cell are affected by NE depletion, and this possibility may partially explain why follicle expansion, Ab production by primary B cells, and germinal center formation were less evident after Ag exposure in NE-depleted mice compared with those in NE-intact controls.

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