FUNCTIONAL ANALYSIS OF INFLUENZA-SPECIFIC HELPER T CELL CLONES IN VIVO

T Cells Specific for Internal Viral Proteins Provide Cognate Help for B Cell Responses to Hemagglutinin

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Influenza virus is a negative-stranded RNA virus whose genome consists of eight single-stranded segments which encode at least 10 polypeptides (1). These include four major structural proteins: the hemagglutinin (HA)[1] and neuraminidase (NA), antigenically variable surface glycoproteins; and the nucleoprotein (NP) and matrix (M), which are antigenically conserved internal proteins.

Both cellular and humoral immune responses are important in the host defense to influenza infection. Antibodies to the HA have been shown to be critical for virus neutralization (2) and for protection against subsequent infection (3), whereas cytotoxic T cells play a role in the clearance of the virus in an ongoing infection (4–8). Both of these responses appear to be Th cell dependent (9, 10). Hence, the activation of Th cells may be critically important to the magnitude of the host antiviral immune response.

Previous studies have established that Th cells can support antigen-specific B cell responses through two distinct pathways (reviewed in 11 and 12). One, referred to as cognate help, is thought to result from a direct T–B interaction, while the other, referred to as noncognate or bystander help, is mediated through factors released from activated T cells that act nonlocally upon antigen-activated B cells. Classic cognate help appears to require that the antigenic determinants recognized by Th and B cells be covalently linked on the same antigen, thus mediating (or facilitating) a direct T–B interaction. In contrast, bystander help has no such requirement and therefore occurs in situations in which T and B cell determinants are present on noncovalently linked antigens. The type of help operative in an antigen-specific B cell response appears to depend primarily on the population (13) and/or activation state (14) of the B cells preferentially expanded under the conditions of the experiment.

Special rules may apply, however, for T–B interactions in antiviral responses. Thus, Russell and Liew (15, 16) reported that mice primed with influenza virus cores (devoid of viral surface proteins HA and NA) or purified M protein

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[1] *Abbreviations used in this paper:* CAS, Con A supernatant; HA, hemagglutinin; HAU, hemagglutination unit; M, matrix; MID_{50}, 50% mouse infection dose; NA, neuraminidase; NP, nucleoprotein.

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exhibited an enhanced anti-HA antibody response when challenged with intact virus, but not when challeged with a mixture of viral cores and purified HA. Additionally, Lamb et al. (17) found that a human Th clone specific for influenza virus M protein provided help for secondary HA-specific B cell responses in vitro upon stimulation with intact virus, but not with a mixture of isolated M protein and HA. Since M and HA are not covalently linked within the virion, yet mediated a cognate T–B interaction, these authors termed the observed help as intermolecular/intrastructural (18).

While these studies provided evidence that Th to the internal M protein could help B cells respond to HA, the question remained whether this type of intermolecular help could be as effective as the classic intramolecular help in terms of extent of enhancement and isotype switch. Additionally, the role of Th specific for the NP in this response remained unknown. We addressed these questions in the present study by comparing the effects of an HA- M-, and NP-specific Th clone on the antibody response of nude mice after infection with A/PR/8/34 (PR8) influenza virus. We show that nude mice adoptively transferred with individual Th clones exhibit a strongly accelerated antiviral antibody response upon infection, directed predominantly against the HA. Further, Th cells specific for the internal proteins M and NP are as effective in vivo as an HA-specific Th clone in providing help for HA-specific B cells during a primary response. In each case, the observed help follows the rules of cognate interaction and thus supports the existence of intermolecular/intrastructural T–B interaction in the anti–influenza virus response.

Materials and Methods

Viruses and Viral Antigens. All viruses used in this study were grown in the allantoic cavity of embryonated hen eggs. The titers were determined as described previously (19) and are expressed in hemagglutination units (HAU) per milliliter. The abbreviations, full designations, and origins of the viruses used here have been described in detail elsewhere (20). Viral proteins were prepared and their purity was assessed as described (21).

Production, Cloning, and Maintainance of Th Clones. The generation and cloning of the V1.2 Th cell clone have been described in detail previously (22). The TL1/3.1 and T2.5-26 cell lines were isolated from the spleen and inguinal lymph nodes, respectively, of BALB/c mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) 8 d after priming in the hind footpads with ~50 HAU of PR8 in CFA (Difco Laboratories Inc., Detroit, MI). The lines were maintained by alternate cycles of antigen stimulation followed by growth in medium supplemented with 2% IL-2-containing supernatant (from 24-h Con A–stimulated rat splenocytes). Antigen stimulation was achieved by incubating the T cell lines at $1–2 \times 10^5$ cells/ml with PR8 (1–5 HAU/ml) and 2×10^6 irradiated (2,200 rad) syngeneic spleen cells/ml in Iscove's complete medium (23) supplemented with 0.2% BSA (Sigma Chemical Co., St. Louis, MO), 0.5% lipids (Boehringer Mannheim Biochemicals, Indianapolis, IN) and 2% FCS (KC Biological, Inc., Lenexa, KS). After 4–6 wk, the lines were cloned by limiting dilution. These clones have been stable in culture for >9 mo.

Proliferation Assay. Cloned T cells, 7–10 d after antigen stimulation, were passed over Ficoll-Hypaque and cultured at 2×10^4 cells/well with 4×10^5 irradiated spleen cells per well and several concentrations of antigen in a final volume of 0.2 ml of Iscove's plus 2% FCS. These cultures were incubated in flat-bottomed microtiter plates (Falcon Labware, Oxnard, CA) at 37°C and 7% CO_2. After 72 h, they were pulsed with 0.2 μCi of [^{3}H]-thymidine (New England Nuclear, Boston, MA) and harvested onto glass fiber filters 12–18 h later. [^{3}H]Thymidine incorporation was measured by liquid scintillation spectroscopy.

Determination of Ia Restriction. 2×10^4 cloned T cells were cultured with PR8 (5

HAU/well) and 5×10^4 L cells expressing either the cloned I-A^d or I-E^d genes (generously provided by Dr. Ronald Germain, National Institutes of Health, Bethesda, MD) in a final volume of 0.2 ml. 100 μl of supernatant from these cultures were harvested after 48 h and added to 100 μl of the IL-2-dependent CTLL line (10^4 cells/well). These cultures were then pulsed after 24–48 h and were harvested as described above.

Assay for Th Activity In Vitro. Splenocytes from BALB/c mice, primed by intraperitoneal injection of PR8 (2,000 HAU) 6–8 wk earlier, were depleted of T cells by treatment with anti-Thy-1.2 (13.4.9) and guinea pig complement (Gibco Laboratories, Grand Island, NY). These cells were cultured at 2×10^5 cells/well with 10^4, 10^3, or 10^2 cloned T cells (treated with mitomycin c [Sigma Chemical Co.] before assay) and 50–0.5 HAU/well of virus. After 5 d, the cultures were washed and the medium was replaced. After two additional days of culture, the supernatants were harvested, diluted 1:5 or 1:25, and assayed for antiviral antibodies in a solid-phase RIA using PR8 as the immunoadsorbent (24) and an iodinated monoclonal rat anti-C_κ (25) as the developing reagent.

Virus Infection In Vivo. BALB/c mice and BALB/c *nu/nu* mice (Harlan Sprague Dawley, Inc.) were infected with aerosolized PR8 (Airborne Infection Apparatus, No. A42, Tri-R Instruments, Inc., Rockville Center, NY) corresponding to 100 MID_{50} (50% mouse infectious dose). ~18 h later, the *nu/nu* mice were reconstituted by intravenous injection with $1–5 \times 10^6$ cloned T cells. Sera from the animals were collected by retroorbital bleeding at various intervals thereafter. For the coinfection experiment, mice were exposed sequentially to aerosolized PR8 and HK or B/Lee, all corresponding to 100 MID_{50}.

Analysis of Serum Antiviral Antibody Titers. Antiviral antibodies in the serum were determined in an RIA (24) using PR8 (20 HAU/well), HK (20 HAU/well), or B/Lee (20 HAU/well) as the immunoadsorbents and iodinated monoclonal rat anti-C_κ as the developing reagent. Sera were diluted from 1:100 to 1:12,500 in HAS (PBS, pH 7.2, 0.04% NaN_3) plus 1% BSA. The specificity of the anti-PR8 antibodies was also determined by RIA, using bromelain-solubilized HA (200 ng/well) or viral cores (300 ng/well) as the immunoadsorbents. Both the HA and viral cores were obtained by treatment of the virus with bromelain as described by Brand and Skehel (26). Isotype analysis was performed using isotype-specific rabbit anti-mouse antisera (27), or monoclonal rat anti-mouse (CooperBiomedical, Inc., Malvern, PA) reagents followed by an iodinated goat anti-rabbit (27) or affinity-purified mouse anti-rat antiserum (Pel-Freeze Biologicals, Rogers, AR), respectively. In all cases, the antibody titer was expressed as the $\log_{10}$ of the reciprocal dilution giving equivalent levels of cpm as the lowest dilution of preimmune serum tested in the assay.

Results

Specificity and Characterization of Th Cell Clones. The T cell clones were generated as described in Materials and Methods and were maintained in culture by alternate passage with irradiated spleen cells and antigen or with rat Con A supernatant (CAS). All assays were performed with T cells that had been cultured with CAS for 3–5 d and had been purified over a Ficoll-Hypaque gradient before use.

The specificity of the V1.2 has been described previously (22) and is shown in Table I. This clone recognizes the HA of the H1 subtype and is restricted to the H-2^d haplotype. It is also alloreactive to H-2^k. Using synthetic peptides, the fine specificity of the clone has been further mapped to residues 111–119 on the HA1 molecule (unpublished observations). This region has been designated site 1 and appears to be an immunodominant Th cell determinant for BALB/c mice.

The antigen specificity and H-2 restriction of the TL1/3.1R1 and T2.5-26 clones were determined by [^{3}H]thymidine incorporation in a proliferation assay. As shown in Table I, the T2.5-26 clone proliferates to type A viruses of the H1

TABLE I
Antigen Specificity of Th Clones

Antigen	[^{3}H]Thymidine uptake (cpm)		
	V1.2	TL1/3.1	T2.5-26
Medium	6,707	443	4,068
Con A	80,465	90,939	52,087
PR8 (H1N1)	148,848	38,062	235,126
B/Lee	2,602	555	4,692
HK (H3N2)	2,764	291	31,925
Japan (H2N2)	913	525	40,313
BHA	159,541	411	5,800
BNA	8,640	233	4,230
M	5,241	638	146,886
NP	6,647	36,476	5,547

T cells were assayed 7–10 d after antigen stimulation and were passed over a Ficoll-Hypaque gradient before use. 2×10^4 T cells were added per well with 4×10^5 irradiated spleen cells (2,200 rad) and antigen, either in the form of virus grown in allantoic fluid or purified viral proteins, at a concentration determined to be optimal (PR8, B/Lee, HK, and Jap at 1–5 HAU/ml; BHA, BNA, M, and NP at 100–200 ng/ml; Con A at 5 μg/ml). The cells were cultured for 3 d, pulsed with 0.2 μCi of [^{3}H] thymidine, and were harvested 12–18 h later. The values shown represent the mean [^{3}H]thymidine uptake of 3–4 replicate wells.

(PR8), H2 (Jap), and H3 (HK) subtypes but not to an unrelated type B virus (B/Lee). The TL1/3.1 clone, however, is stimulated by type A viruses of only the H1 subtype. Additionally, both clones are restricted to the H-2^d haplotype and show no alloreactivity to either H-2^b or H-2^k (data not shown). Using purified viral proteins (Table I), as well as a panel of recombinant viruses possessing assortments of genes from the PR8 and HK parental viruses in the case of TL1/3.1 (data not shown), we mapped the specificity of the T2.5-26 and TL1/3.1 clones to M and NP, respectively. The precise region recognized on either the NP or M protein is unknown. The TL1/3.1R1, however, is stimulated by all natural influenza virus variants of the H1 subtype isolated between 1931 and 1980 (data not shown), unlike the cytotoxic T cell clones described by Townsend et al. (28).

We further characterized the H-2 restriction of each of the clones by using L cells transfected with genes encoding either the I-A^d or I-E^d molecule (29). Since the clones do not proliferate to antigen presented by the L cells (possibly due to the lack of appropriate factor secretion by the L cells), stimulation was determined by IL-2 production. Both the T2.5-26 (M-specific) and TL1/3.1 (NP-specific) are shown to be restricted to I-A^d, while the V1.2 (HA-specific) is restricted to I-E^d (Table II). Additionally, all three of the clones express the Ly-1$^+$, Lyt-2$^-$ helper phenotype, as determined by FACS analysis (data not shown).

As expected from their surface phenotype and Ia restriction, the T cell clones could be shown to exhibit helper activity for an antiviral B cell response in vitro (Fig. 1). Anti-PR8 antibodies produced by secondary B cells cultured with or without Th clones and antigen were measured in an RIA. It is evident that production of antiviral antibodies requires the presence of both a T cell clone

TABLE II
Ia Restriction of Th Clones

L cell transfectant	Ia expression	V1.2	TL1/3.1	T2.5-26
RT10.3H2	I-E^d	46,276	1,994	3,220
RT2.3.3H	I-A^d	2,556	23,594	56,930
RT1.1.12	None	2,504	2,043	3,224

Ia restriction of the T cell clones was determined by culturing 2×10^4 T cells with 5 HAU of PR8 and 5×10^4 transfected L cells. Supernatants were harvested after 48 h and added to the IL-2 dependent CTLL line (10^4 cells/well). After 24–48 h, proliferation of the CTLL cells was assessed by [^{3}H]thymidine incorporation. The values represent the mean of triplicate wells.

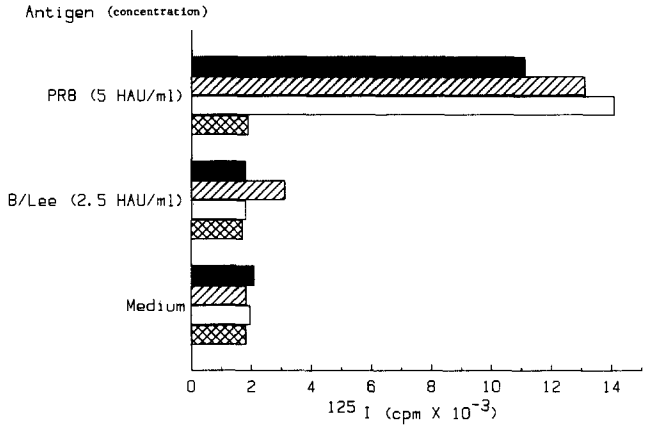

FIGURE 1. In vitro helper function of Th clones. Splenocytes from PR8-primed BALB/c mice were depleted of T cells by anti-Thy-1 and complement, and then were cultured at 2×10^5 cells/well with graded numbers of mitomycin-treated T cell clones V1.2 (■), TL1/3.1 (▨), T2.5-26 (▭) or no T cells (▩) and an optimal dose of antigen for 5 d, at which time the cultures were washed and the medium replaced. After two additional days, the supernatants were harvested and assayed in an RIA (see Materials and Methods). The number of T cell clones that supported an optimal antibody response under these culture conditions was 1,000 cells/well of V1.2 and T2.5-26 and 10,000 cells/well of TL1/3.1. The results above represent the mean cpm seen with a 1:5 dilution of culture supernatant obtained from triplicate wells.

and the appropriate virus as antigen, since a significant level of antibodies is not detected in the absence of either.

Th Clones Exhibit Helper Function for B Cell Responses In Vivo. Since the T cell clones could be shown to exhibit helper function in vitro, we next examined the effect of the clones on the antiviral B cell response in vivo. Athymic *nu/nu* mice were infected with aerosolized PR8 and ~18 h later were injected i.v. with 1–5 $\times 10^6$ T cells. The serum antibody titers were then compared among animals receiving either the NP-specific, HA-specific, M-specific clone, TL2/4.5R7 (specific for a component in the allantoic fluid of embryonated hen eggs in which the virus is grown) or no T cells. The TL2/4.5R7 clone served as a control for nonspecific effects resulting from the adoptive transfer of cloned T cells. The titer of PR8-specific antibodies in the serum was determined in an RIA.

Nude mice that received either the control TL2/4.5 clone or no T cells did not produce anti-PR8 antibodies (Fig. 2), thus showing the requirement of virus-

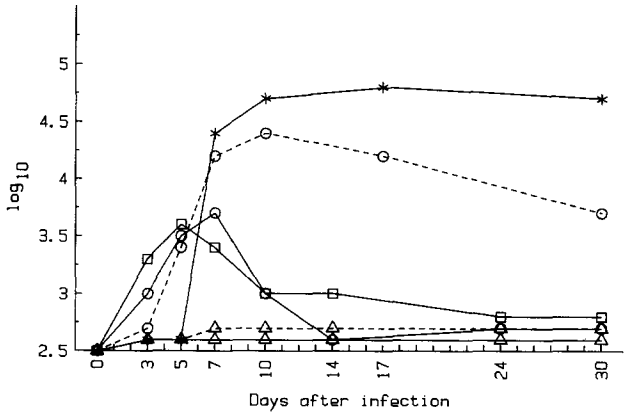

FIGURE 2. Kinetics of the serum anti-PR8 antibody response in reconstituted nude mice. The antibody titer in the serum of BALB/c mice (*—*), or nude mice reconstituted with the NP-specific (○—○), HA-specific (□—□), M-specific (○- -○), control clone (△—△), or no T cells (△- -△) was determined in an RIA using PR8 (20 HAU/well) as the immunoadsorbent and iodinated rat monoclonal anti-C_κ as developing reagent. Sera were diluted from 1:100 to 1:12,500 in HAS plus 1% BSA, and the titer was expressed as the log 10 of the reciprocal dilution that gave 2,000 cpm.

specific T cells in the antibody response to influenza (9, 30, 31). In contrast, mice receiving either the NP-specific, M-specific, or HA-specific clone could mount a significant antibody response to the virus. This response was detectable as early as day 3 after infection, and peaked on days 5–7. The kinetics of the response were accelerated by ~3 d in comparison to those seen in normal BALB/c mice.

Although an antibody response was generated in nude mice reconstituted with either the HA-, M-, or NP-specific clone, we saw differences in the titer and duration of the antibody response supported by the individual clones. Transfer of the HA- or NP-specific clone resulted in similar levels of serum antibodies in the nude mice. The titer, however, was ~10-fold lower than that found in normal animals. The antibody response in these nude mice was also more transient than in normal mice, where a high titer was detected as late as 30 d after infection. In nude mice reconstituted with the NP-specific clone, the titer usually dropped to background levels around day 14. The sharp decrease by day 14 was also seen in nude mice reconstituted with the HA-specific clone, although a low level of antibodies was still present on day 30 (seen in three individual experiments). Nude mice reconstituted with the M-specific clone generated an antibody response most comparable to that seen in normal mice. The antibody titer peaked at a level approximately sevenfold higher than that supported by the NP- or HA-specific clones, and it persisted at high levels throughout the experiment.

Specificity and Isotype of Antibodies Generated in Reconstituted Nudes. It has been shown that antibodies to the HA are important for both protection against (3) and neutralization of (2) influenza virus. To determine whether the levels of anti-HA antibodies supported by the NP-, M-, or HA-specific Th clone differed from each other, the sera were further tested in an RIA for the titer of antibodies specific for HA or viral core proteins (mainly NP and M). As shown in Fig. 3*A*, roughly equivalent levels of HA-specific antibodies were detected in the sera of

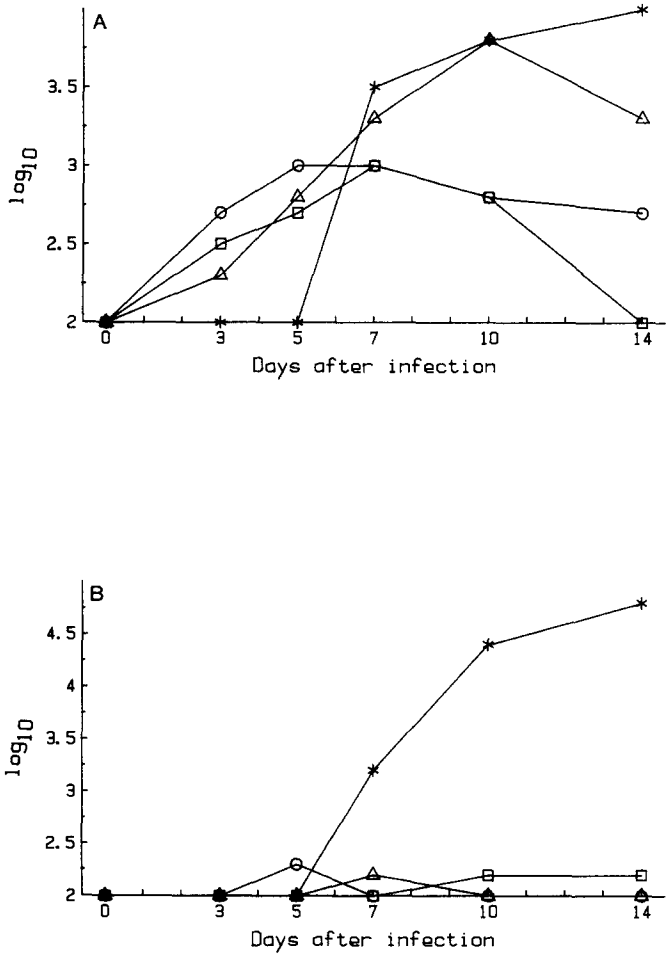

FIGURE 3. Comparison of the specificity of the antiviral antibodies supported by the Th clones in nude mice. Antibodies directed against the HA (*A*) or internal viral components M and NP (*B*) were detected in an RIA using bromelain-solubilized HA at ~200 ng/well or viral cores at ~300 ng/well as the immunoadsorbents. The sera from BALB/c mice (*—*), or nude mice reconstituted with the HA-specific (○), NP-specific (□), or M-specific (△) clone were assayed as described in the legend of Fig. 2. The titers are expressed as the $\log_{10}$ of the reciprocal dilution that gave 1,500 cpm using HA as the immunoadsorbent, or 2,000 cpm using viral cores.

nude mice reconstituted with the HA- or NP-specific Th clone. Again, the titer of the sera from nudes reconstituted with the M-specific clone was significantly higher (approximately sevenfold) and was comparable to that seen in normal BALB/c mice. These results were confirmed by testing the sera in a hemagglutination inhibition assay (data not shown).

In contrast to normal BALB/c mice, which had relatively high titers of antibodies to the internal viral proteins M and NP, little or no antibody to these proteins was detected in the sera of reconstituted nude mice (Figure 3*B*). Thus, the antibody response in the nude mice appeared to consist almost entirely of anti-HA antibodies, regardless of the specificity of the helper clone with which they were reconstituted.

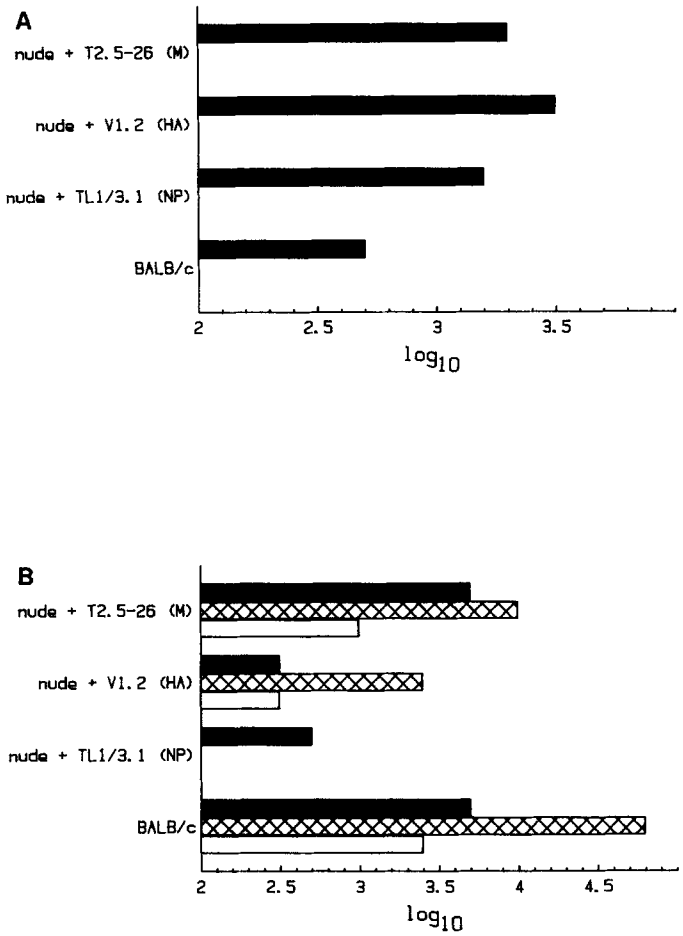

FIGURE 4. Isotype composition of the anti-PR8 antibodies generated in reconstituted nude mice. Antiviral antibodies in the serum of the reconstituted nude mice were tested in an RIA 5 d (*A*) and 14 d (*B*) after infection for antibodies of the IgM (■), IgG (▩), and IgA (▭) isotypes (see Materials and Methods). The titers are expressed as the $\log_{10}$ of the reciprocal dilution of sera giving 1,500 cpm for IgM antibodies and 2,000 cpm for IgG and IgA antibodies.

The isotype composition of the antiviral antibody response 5 and 14 d after infection is shown in Fig. 4. In normal BALB/c mice, only IgM antibodies were detected 5 d after infection. By day 14, however, significant levels of IgA and IgG antibodies were also detected, showing the isotype switch that normally occurs during a primary immune response. We also saw this switch in nude mice reconstituted with the HA- and M-specific clones. In contrast, transfer of the NP-specific clone resulted in the production of antibodies only of the IgM isotype throughout the response. This failure to switch may in part explain the shorter duration of HA-specific antibodies in the nudes receiving the NP clone. Additionally, the ability to generate antibodies of the IgM, IgG, and IgA isotypes in nude mice receiving the HA- and M-specific clones suggests that in terms of isotype composition, the reconstitution of the antibody response in these animals is complete.

TABLE III

Requirement for Cognate Help in Generation of Antiviral Antibodies

Mice	Specificity of transferred Th	Infection with	Antibody titer (log_{10})		
			PR8	HK	B/Lee
Normal	—	PR8 + HK	0.7	0.7	—
Nude	—	PR8 + HK	0.1	0	—
Nude	Anti-M (PR8,HK)	PR8 + HK	0.5	0.6	—
Nude	Anti-HA (PR8)	PR8 + HK	0.6	0	—
Normal	—	PR8 + B/Lee	1.5	—	1.2
Nude	—	PR8 + B/Lee	0	—	0.1
Nude	Anti-NP (PR8)	PR8 + B/Lee	0.8	—	0.1
Nude	Anti-HA (PR8)	PR8 + B/Lee	1.1	—	0

Sera were obtained from animals 7 d after coinfection with either PR8 and HK or PR8 and B/Lee viruses. The titer of antiviral antibodies produced was determined in an RIA using purified PR8 (20 HAU/well), purified B/Lee (20 HAU/well), or purified HK (20 HAU/well) as the immunoadsorbents and iodinated rat monoclonal anti-C_κ as the developing reagent. The antibody titer = (the log_{10} of the reciprocal dilution of day 7 serum giving 2,000 cpm) − (the log_{10} of the reciprocal dilution of preimmune serum giving 2,000 cpm). In the experiments shown above, a 1:400 dilution of preimmune serum gave 2,000 cpm.

Requirement of Cognate T–B Interaction in Response. The data presented thus far show that the antiviral antibody response in nude mice requires virus-specific Th cells and that an NP and M–specific Th clone is as effective as an HA-specific Th clone in providing help for a primary IgM response to the HA. We next wanted to determine whether the observed help resulted from linked recognition of viral antigens by the T and B cells (cognate interaction), or whether it was mediated by factors released from the activated Th clones which then acted in a nonspecific way on any antigen-triggered B cell (noncognate or bystander activation). To distinguish between these two possibilities, nude mice were coinfected with either PR8 and HK or PR8 and B/Lee, and reconstituted with individual Th clones. Sera were then examined at various time points for antibodies specific for each of the infecting viruses. As shown in Table III, nude mice receiving the M-specific clone, which recognizes PR8 and HK, produced antibodies to both viruses by day 7. Reconstitution of nude mice with the Th clone specific for the HA of PR8, however, elicited an antibody response only to PR8 in animals coinfected with either PR8 and B/Lee or PR8 and HK. Transfer of the NP-specific Th clone, which recognizes only PR8, also failed to provide bystander help to B/Lee-specific B cells in animals coinfected with PR8 and B/Lee. These results indicate that induction of the antiviral antibody response supported by the individual Th clones in this experimental system requires a linked T–B interaction. It remains possible, however, that transfer of higher numbers of these particular clones or transfer of different Th clones may result in significant bystander activation.

Discussion

There are three main findings that emerge from this study: First, nude mice, after adoptive transfer of individual virus-specific Th clones, can generate an antiviral antibody response to pulmonary influenza virus infection. This response

is accelerated by ~3 d, compared with the primary antibody response of normal mice and with two of the three clones studied, an isotype switch similar to that which occurs during a normal antibody response is seen. Second, Th cells specific for the internal viral proteins, M and NP, are as effective as an HA-specific Th clone in supporting a primary B cell response to the HA. Third, the type of help seen in this experimental system requires a cognate T–B interaction whether or not the determinants recognized by the Th and B cells are located on the same viral protein or on different, noncovalently linked proteins assembled within the same virus particle.

The evidence that a cognate interaction is required to generate an anti-HA response comes from the results of the coinfection of reconstituted nude mice with either PR8 and HK or PR8 and B/Lee. In this case, an antibody response was generated only to PR8 in nude mice receiving the HA- or NP-specific Th clones. In nude mice reconstituted with the M-specific clone, which recognizes both PR8 and HK, antibodies to both viruses were produced. Since no bystander activation was seen against a virus that was not recognized by the Th clone transferred, it appears that the antibody response supported in this experimental system by the individual Th clones examined is entirely due to cognate help. This is the case even if the Th and B cell recognize different PR8 viral proteins, therefore supporting the concept that effective intrastructural/intermolecular help occurs in this antiviral antibody response.

The phenomenon of intrastructural/intermolecular help can be explained as follows: Current evidence indicates that B cells can function as APCs (32–42). Although high concentrations of antigen are required for B cells to take up antigen nonspecifically for presentation to T cells, B cells have been shown (39–42) to present very efficiently the antigen for which they are specific, probably due to their ability to interact with and take up this antigen via their surface Ig receptors. This promotes a direct antigen-specific T–B interaction and underlies the requirement for the covalent linkage of Th and B cell antigenic determinants seen during classic cognate help. In the influenza system, however, an HA-specific B cell that has interacted with and taken up an intact virus particle can present on its surface processed viral proteins other than HA, and thereby can receive direct help from T cells specific for internal as well as surface viral proteins. By this mechanism, intermolecular/intrastructural help would occur only when the intact virus serves as antigen, a requirement seen in previous studies (15–17). Note that efficient uptake of intact virus (but not free internal proteins) by B cells can be mediated via sialic acid–containing surface receptors (L. C. Eisenlohr, W. Gerhard, and C. J. Hackett, manuscript submitted for publication), and therefore does not require initial focusing via surface Ig. A subsequent surface Ig–antigen interaction, however, appears to be required to render HA-specific B cells responsive to direct T cell help, since antibodies to the internal viral proteins are not seen.

Although helper function for an HA-specific B cell response could be mediated by either an HA-, M-, or NP-specific clone, we saw differences in the antibody responses supported by the individual clones. Specifically, the magnitude and duration of the response supported by the M-specific clone were significantly greater than those seen in nude mice reconstituted with either the HA- or NP-

specific clones. Additionally, the response supported by the NP-specific clone consisted exclusively of antibodies of the IgM isotype, whereas a definite switch to the IgG and IgA isotypes occurred in the presence of both the M- and HA-specific clones.

There are several possible explanations for these differences. First, the factors secreted by the individual clones may vary both qualitatively and quantitatively. There is evidence that IL-2 (43–46), IFN-γ (47), as well as B cell growth and differentiation factors (48) are important signals for the activation and expansion of B cells to antibody-secreting cells during a direct T–B interaction. Second, differences in the expansion and/or survival of the Th clones in vivo could account for the results we saw. Third, the specificity of the clone could play a role in its efficiency for helping HA-specific B cells. It is possible, for example, that HA-specific B cells, after uptake of intact virus, process the M protein (which is the most abundant viral structural protein) more efficiently than NP or HA, and therefore, focus M-specific Th more effectively than NP- or HA-specific Th cells. We feel that this possibility is the least likely because no significant difference can be detected in the efficiency of processing of M and HA by B cell lymphomas (A20, L10) in vitro (L. Eisenlohr and C. Hackett, personal communication). Experiments are currently underway, however, to further discriminate among these possibilities.

Finally, there are several striking differences between the antibody response supported in the reconstituted nude mice and that seen in normal BALB/c mice. The kinetics of the antibody response elicited in the reconstituted nudes is accelerated by ~3 d in comparison with normal animals. The simplest explanation of this finding is that the presence of an expanded population of antigen-specific T cells can accelerate the expansion and differentiation of primary antigen-specific B cells. Previous studies by Miller and Sprent (49), using irradiated mice, however, showed that antigen-primed T cells could not enhance the antibody response of unprimed B cells to fowl gamma globulin. Thus, a lack of regulatory mechanisms such as T suppressor cells or a difference in the population of B cells present in nude mice (50) may also account for the shift in kinetics we saw.

In contrast to BALB/c mice which produce comparable levels of antibodies to both the HA and the internal viral proteins, the antibody response in the reconstituted nude mice is directed almost entirely to the HA. This does not appear to be due to an inherent inability of nude mice to produce antibodies to M and NP, since antibodies to these proteins can be detected in supernatants of *nu/nu* B cells cultured with the Th clones and PR8 in vitro (data not shown). Thus, the HA, although clearly a T cell–dependent antigen, may require less T cell help to effect B cell activation than the NP and M proteins (51). Alternatively, if interaction of antigen with surface Ig is a precondition for effective T–B interaction, M- or NP-specific B cell responses may occur only when these proteins are present in free form, e.g., upon lysis of infected cells. Thus, if the adoptively transferred T cells were functional for only a short time, B cells specific for proteins appearing later during infection may not receive adequate help. This is supported by preliminary experiments showing that transfer of the NP-specific Th clone 3 d (instead of 18 h) after infection provides effective help for B cells responding to internal viral proteins.

Although in vivo function of murine class II–restricted T cell clones specific for sheep erythrocytes (52) and myelin basic protein (53) has been described, this study is to our knowledge the first demonstration of helper function by virus-specific Th clones in vivo. That this system reflects T–B interactions that occur in a normal antibody response is evidenced by the observed requirement for cognate help. Additionally, recent experiments have shown that nude mice reconstituted with the Th clones show a significant reduction, and in some cases, complete clearance of virus from the lung (P. A. Scherle and W. Gerhard, manuscript in preparation). Further analysis of these clones should help elucidate the mechanisms involved and the factors required for generation of a successful antiviral immune response.

Summary

We compared the effects of adoptively transferred Th cell clones specific for the influenza hemagglutinin (HA), matrix (M), or nucleoprotein (NP) on the antibody response of nude mice infected with A/PR/8/34 influenza virus. We show that the production of antibodies to the HA absolutely requires the presence of virus-specific Th cells. Further, transfer of a Th clone specific for the internal proteins, M or NP, was as effective as was transfer of an HA-specific clone in supporting an antibody response to the HA. With each of the clones, the kinetics of the response were accelerated by ~3 d compared with the antibody response of normal BALB/c mice. The HA- and M-specific clones supported an isotype switch from IgM to IgG and IgA similar to that which occurs during a normal antibody response. Finally, as shown by coinfection experiments, the response required a cognate T–B interaction whether the determinants recognized by the Th and B cell are located on the same viral protein or on different viral proteins within the same virus particle. The implications of these findings for understanding the T–B interactions that occur during an effective antiviral antibody response are discussed.

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