JOURNAL OF VIROLOGY, Nov. 1994, p. 6863–6870
0022-538X/94/$04.00+0

Vol. 68, No. 11

Analysis of the Primary T-Cell Response to Sendai Virus Infection in C57BL/6 Mice: CD4$^+$ T-Cell Recognition Is Directed Predominantly to the Hemagglutinin-Neuraminidase Glycoprotein

GEOFFREY A. COLE,[1] JACQUELINE M. KATZ,[2]† TWALA L. HOGG,[1] KEVIN W. RYAN,[2,3] ALLEN PORTNER,[2,3] AND DAVID L. WOODLAND[1,3]*

Departments of Immunology[1] and Virology,[2] St. Jude Children's Research Hospital, Memphis, Tennessee, 38105, and Department of Pathology, University of Tennessee, Memphis, Tennessee 38163[3]

Received 9 June 1994/Accepted 26 July 1994

Sendai virus infection of C57BL/6 mice elicits a strong CD4$^+$ and CD8$^+$ T-cell response in the respiratory tract. To investigate the specificity of the CD4$^+$ T-cell response, a panel of hybridomas was generated from cells recovered from the respiratory tracts of infected mice. Using vaccinia virus recombinants expressing individual Sendai virus proteins, we found that the majority of these hybridomas (34 of 37) were specific for the hemagglutinin-neuraminidase (HN) glycoprotein. The hybridomas were then analyzed for reactivity to a set of overlapping peptides spanning the entire length of the hemagglutinin-neuraminidase glycoprotein. At least five *H-2* I-A^b-restricted epitopes were defined in HN. The strong bias toward recognition of class II epitopes derived from a single viral protein contrasts with T-cell recognition of epitopes of several proteins in influenza A virus as found previously by others.

T cells are a central component of the immune response to acute viral infection. These cells recognize viral antigens as small peptides bound to major histocompatibility complex (MHC) class I and class II molecules through a clonally distributed T-cell antigen receptor (TCR) (28). Class I-restricted CD8$^+$ T cells, assayed as cytotoxic T lymphocytes (CTL) in vitro, are generally considered to be the principal effectors involved in elimination of virus-infected cells (16). Class II-restricted CD4$^+$ T cells are involved in the induction of antibody responses (15, 31, 38). The intracellular compartments in which protein fragments are generated and loaded onto class I and class II molecules are different (18, 35). Generally, class I-associated antigenic peptides derive from cytosolic proteins (5, 51, 52), whereas class II-associated peptides derive from internalized extracellular antigens or endogenous proteins that access the endosomal/lysosomal compartment (5, 12, 36). Development of strategies for vaccination against viruses is dependent on identification of viral epitopes recognized by both CD8$^+$ and CD4$^+$ T cells elicited during virus infection.

Murine parainfluenza type 1 virus, Sendai virus, is a natural respiratory pathogen of mice with substantial structural and serological relatedness to the human parainfluenza type 1 viruses that can cause severe lower respiratory tract disease in children (9, 19, 20). Sendai virus-specific CD8$^+$ T cells recovered from the respiratory tracts of infected C57BL/6 (B6) mice show CTL activity directed against a single *H-2K*^{b}-restricted immunodominant epitope on the viral nucleoprotein and appear to play a major role in viral clearance in immunocompetent mice (13, 21, 27). However, the precise role of CD4$^+$ T cells in recovery from this virus infection is unclear. In vivo depletion of CD4$^+$ T cells has little effect on the induction of Sendai virus-specific CTL, virus clearance, or survival in B6 mice (19). In contrast, CD8$^+$ T-cell-depleted B6 and class I negative (B6 × 129) β_2m (−/−) mice exhibit delayed virus clearance and increased mortality following Sendai virus infection (21). Nonetheless, these animals are capable of eliminating virus (21). Although virus-specific class II-restricted CTL have been found in class I negative β_2m (−/−) mice, it is not clear whether these CD4$^+$ T cells play a role in virus clearance through cell-mediated lysis or another mechanism such as the provision of T help for antibody production (21, 24, 25).

The specificity of Sendai virus-specific CD4$^+$ T cells has not been previously defined. Because these T cells can play an auxiliary role in viral clearance in this model, it was of interest to characterize the specificity and repertoire of the CD4$^+$ T cells elicited by Sendai virus infection. We have now generated a panel of T-cell hybridomas from CD4$^+$ T cells isolated from the respiratory tract of B6 mice with primary Sendai virus pneumonia. The specific viral components recognized by these MHC class II-restricted hybridomas have been identified by using both recombinant vaccinia viruses (Vac recombinants) expressing individual Sendai structural proteins and a panel of synthetic peptides.

MATERIALS AND METHODS

Mice. Female B6 mice were purchased from the Jackson Laboratory, Bar Harbor, Maine, and maintained under specific-pathogen-free conditions until infected with virus at 8 to 10 weeks of age.

Virus infections. The Enders strain of Sendai virus (39) was grown and titrated in the allantoic cavity of 10-day-old embryonated eggs (21). Virus stocks were stored at −70°C as allantoic fluid. B6 mice were anesthetized by intraperitoneal injection with Avertin (2,2,2-tribromoethanol) and infected intranasally (i.n.) with 30 μl of phosphate-buffered saline containing 100 to 300 50% egg infective doses of Sendai virus. All mice were held in a biosafety level 3 facility and were seronegative for Sendai virus before infection.

Synthetic peptides. A set of overlapping 17-amino-acid-long peptides spanning the entire 575-amino-acid length of the

* Corresponding author. Phone: (901) 531-2462. Fax: (901) 522-8570.

† Present address: Influenza Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333.

predicted Sendai virus Enders strain hemagglutinin-neuraminidase (HN) glycoprotein (19) was synthesized by Chiron Mimotopes (Clayton, Australia). The peptides all shared a non-HN C-terminal glycine residue and overlapped by six residues, to give a total of 97 peptides. Sendai virus nucleoprotein peptide 321-336 ($NP_{321-336}$) has been previously described (13, 27).

Vac recombinants. Full-size cDNA clones of each Sendai virus gene (F, HN, NP, M, and P) were inserted into plasmid pSC11 (8). Cultured TK^-143 cells were plasmid transfected and infected with vaccinia virus (Western Reserve strain). Vac recombinants capable of expressing the cloned Sendai virus genes were selected and propagated by established methods (8, 37). Recombinant proteins produced by Vac recombinant-infected tissue culture cells were indistinguishable from their authentic Sendai virus counterparts with respect to electrophoretic mobility and reactivity with specific monoclonal antibodies (MAbs) (data not shown).

T-cell proliferation assay. B6 mice were infected i.n. with Sendai virus, and spleens were harvested 4 weeks later. Spleen cells were cultured in triplicate at 4×10^5 cells per well for 4 days in the presence of UV-irradiated infectious allantoic fluid or purified protein. Proliferation was measured in the final 18 h of incubation by incorporation of 0.5 μCi of [^{3}H]thymidine (Amersham, Arlington Heights, Ill.) per well. Sendai virus HN glycoprotein tetramers (used as protein antigen) were prepared and purified as described (49).

In vitro stimulation of BAL populations. Bronchoalveolar lavage (BAL) populations were recovered from Sendai virus-infected mice at 11 days after i.n. infection as previously described (1). BAL cell suspensions were depleted of macrophages by adhering cells (10^6/ml) to plastic petri dishes for 90 min at 37°C. Sendai virus-infected B6 splenic stimulator cells were prepared as previously described (13). BAL cell suspensions were cultured at 10^6 cells per ml with 10^6 stimulator cells per ml in six-well culture plates (Costar, Cambridge, Mass.) for 4 days in the presence of 30% interleukin-2 (IL-2) supernatant. IL-2-containing supernatants were generated and quantitated as previously described (17).

T-cell hybridomas. In vitro-restimulated BAL populations were prepared for fusion as previously described (13). Activated cells were fused with the TCR-negative variant of the T-cell thymoma BW5147 (54) and cultured under limiting dilution conditions. Clonal hybridomas expressing αβ TCR were tested for viral specificity in an IL-2 assay.

Flow cytometry. Two-color flow cytometric analysis of BAL populations and hybridomas was done as described previously (14). Briefly, cells were stained with fluorescein isothiocyanate-conjugated anti-CD4 or fluorescein isothiocyanate-conjugated anti-CD8 and biotinylated MAbs specific for individual TCR Vβ determinants, followed with phycoerythrin-conjugated strepavidin (Tago, Burlingame, Calif.). Stained cells were analyzed by using a FACScan and Lysis II software (Becton Dickinson, Mountain View, Calif.).

IL-2 assay. The T-cell hybridomas were assayed for viral specificity by stimulation with Sendai virus-infected cells, Vac recombinant-infected cells, or peptide-treated cells for 24 h. Cells were infected at a multiplicity of infection of 5 with Sendai virus for 18 h or Vac recombinants for 4 h prior to 3,000-R irradiation. For peptide treatment of cells, either $NP_{321-336}$ or the individual HN peptides were added directly to the assay culture at a final concentration of 30 μg/ml. Culture supernatants were assayed for IL-2 as previously described (55). One unit of human recombinant IL-2 (R&D Systems, Minneapolis, Minn.) represents 160 U in our assay. LB15 (*H-2*^{bxd} class I^+ class II^+) B-cell hybridoma cells or EL4 (*H-2*^{b} class I^+) thymoma cells were used as virus-infected stimulator cells. *H-2* I-A^b-transfected L cells, a gift from Ron Germain, National Institutes of Health, were used to present HN peptides, whereas L929-K^b cells were used to present $NP_{321-336}$ (13).

TABLE 1. Proliferative responses of Sendai virus-primed B6 spleen cells to Sendai virus and HN protein

Antigen	Dose/culture	Proliferative response (cpm ± SE)	
		Sendai virus-primed spleen[a]	Unprimed spleen
Sendai virus	1.0 HAU[b]	33,400 ± 664	1,470 ± 92
Allantoic fluid control[c]		3,990 ± 126	1,130 ± 22
Purified Sendai HN	0.1 μg	49,189 ± 452	2,340 ± 91
Medium control		3,045 ± 187	1,118 ± 60

[a] B6 mice were primed with Sendai virus, and their spleen cells were prepared as described in Materials and Methods.
[b] One hemagglutination unit (HAU) is approximately 2,000 50% egg infective doses.
[c] Control allantoic fluid containing influenza type A virus was used at a dilution comparable to that of allantoic fluid containing 1.0 HAU of Sendai virus.

RNA dot blot analysis. The presence of hybridoma TCR α and β transcripts was detected by using a panel of DNA probes specific for Vα and Vβ families as previously described (14). Total cellular RNA, extracted from individual hybridomas by the guanidinium chloride method of Cheley and Anderson (11), was blotted onto replicate nitrocellulose filters and hybridized with ^{32}P-labeled DNA probes specific for members of the TCR Vα families (Vα1 to Vα13) and Vβ elements for which antibodies were not initially available (Vβ1, -4, -10, -12, -15, and -16) and for Cα and Cβ (14). Filters hybridized with Vα probes were washed at low stringency (50°C, 0.1× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) to allow detection of all members of individual Vα families.

RESULTS

Predominant recognition of the HN glycoprotein by Sendai virus-specific hybridomas. Both $CD4^+$ and $CD8^+$ T cells comprise a significant portion of the nonadherent BAL cells in B6 mice acutely infected with Sendai virus (21). The $CD8^+$ CTL population is specific for a single immunodominant *H-2K*^{b}-restricted nucleoprotein epitope, $NP_{321-336}$ (13, 27, 44). However, the specificity of the $CD4^+$ T cells is unknown. One possible target is the virus HN glycoprotein because purified Sendai virus HN glycoprotein induced strong proliferative responses in Sendai virus-primed spleen cells (Table 1). To define the specificity of $CD4^+$ T cells in the respiratory tracts of infected mice, T-cell hybridomas were generated from the BAL of B6 mice 11 d after primary i.n. infection with Sendai virus (13). Cells recovered in the nonadherent BAL are most abundant at this time after infection and consist of both $CD4^+$ and $CD8^+$ populations (13, 21). The hybridoma panel was derived from a single fusion using unseparated nonadherent BAL cells pooled from 10 Sendai virus-infected mice (11).

All TCR^+ hybridomas were initially screened for the ability to secrete IL-2 in response to Sendai virus-infected B6 spleen cells, and 43 Sendai virus-reactive hybridomas were identified. Six of these hybridomas were specific for $NP_{321-336}/K^b$ and are described elsewhere (13). The remaining 37 hybridomas appeared to be MHC class II I-A^b restricted because they recognized LB15 (*H-2*^{bxd} class I^+ class II^+) but not EL4 (*H-2*^{b}

TABLE 2. Analysis of CD4+ T-cell hybridomas for Sendai virus protein specificity

Hybridoma	CD4 expression	IL-2 production (U/ml)[a]						
		L929-K^b $NP_{321-336}$[b]	LB15[c]					
			Uninfected	Sendai virus	Vac-F	Vac-HN	Vac-M	αTCR[d]
B3.1C7	+	−	−	>1,280	−	640	NT[e]	>1,280
B3.1F6	+	−	−	>1,280	−	>1,280	−	>1,280
B3.1F8	+	−	−	640	−	80	NT	640
B3.1G9	+	−	−	>1,280	−	>1,280	−	>1,280
B3.1H4	−	−	−	>1,280	−	640	NT	>1,280
B3.2A8	+	−	−	640	−	80	NT	160
B3.2C5	+	−	−	>1,280	−	640	NT	>1,280
B3.2D4	+	−	−	40	−	−	−	>1,280
B3.2E5	−	−	−	80	−	−	−	320
B3.2F5	+	−	−	>1,280	−	>1,280	NT	640
B3.2F10	+	−	−	>1,280	−	>1,280	−	>1,280
B3.2F11	+	−	−	640	−	640	NT	320
B3.2G4	+	−	−	>1,280	−	160	NT	>1,280
B3.2G9	+	−	−	320	−	80	NT	>1,280
B3.2H3	+	−	−	>1,280	−	>1,280	−	>1,280
B3.3C11	+	−	−	160	−	80	NT	80
B3.3D3	+	−	−	>1,280	−	160	NT	>1,280
B3.3D8	+	−	−	320	−	320	−	40
B3.3E9	−	−	−	>1,280	−	320	−	320
B3.3E11	+	−	−	160	−	80	NT	80
B3.3F6	+	−	−	>1,280	−	−	80	>1,280
B3.3G5	+	−	−	>1,280	−	320	−	>1,280
B3.3G8	+	−	−	160	−	40	−	80
B3.4A5	+	−	−	>1,280	−	>1,280	−	320
B3.4C2	+	−	−	>1,280	−	320	NT	>1,280
B3.4D4	+	−	−	640	−	−	−	>1,280
B3.4D6	+	−	−	>1,280	−	>1,280	NT	>1,280
B3.4F1	−	−	−	>1,280	−	640	NT	>1,280
B3.4F3	+	−	−	>1,280	−	>1,280	−	320
B3.4G2	+	−	−	>1,280	−	>1,280	−	640
B3.4G8	+	−	−	>1,280	−	160	−	320
B3.5B3	+	−	−	20	−	−	−	20
B3.7F5	+	−	−	>1,280	−	−	−	>1,280
B3.7H5	+	−	−	>1,280	−	320	−	160
B3.8D5[f]	+	−	>1,280	>1,280	>1,280	>1,280	NT	>1,280
B3.8G2	+	−	−	640	−	640	−	>1,280
B3.14G5	+	−	−	>1,280	−	640	−	>1,280
B3.2A1[g]	−	80	−	40	−	−	NT	320

[a] Determined by HT-2 assay as described in Materials and Methods. −, IL-2 production below the level of detection (≤10 U/ml).
[b] Peptide was added at 30 μg/ml.
[c] LB15 cells were infected with Sendai virus or Vac recombinants as described in Materials and Methods.
[d] Immobilized anti-TCR β MAb H597-57.
[e] NT, Not tested.
[f] Determined to be Sendai virus/*H-2*^{b} specific (data not shown) and alloreactive on *H-2*^{d}.
[g] An $NP_{321-336}/K^b$-specific $CD4^-$ $CD8^-$ hybridoma included as a specificity control in the assay and derived from the same fusion as the other hybridomas.

class I^+) cells infected with Sendai virus (data not shown). Flow cytometric analysis of these hybridomas demonstrated that 33 of the 37 expressed CD4 (Table 2) and that none expressed CD8 (not shown).

To identify the protein specificity of these hybridomas, they were screened on LB15 cells infected with Vac recombinants expressing the individual Sendai virus HN, F, and M genes. The vast majority of the hybridoma panel (31 of 37) was determined to be HN specific (Table 2). A single hybridoma, B3.3F6, was specific for the M protein, while the protein specificities of the remaining five hybridomas (B3.2D4, B3.2E5, B3.4D4, B3.5B3, and B3.7F5) could not be determined in this assay. B3.4D4 was subsequently lost and not available for further characterization. The protein specificity of B3.7F5 remained undetermined, since additionally it failed to respond to either Vac-NP or Vac-P-infected cells (data not shown). The predominant HN specificity of the hybridoma panel is consistent with the HN glycoprotein-stimulated bulk T-cell proliferation shown in Table 1.

Multiple distinct IA^b-restricted HN epitopes are recognized by HN-specific hybridomas. The HN-specific hybridomas were assayed against a set of 97 overlapping 16-amino-acid-long peptides completely spanning the predicted HN protein sequence (19). MHC class II I-A^b-transfected L cells were used as presenting cells for this assay. Table 3 shows the IL-2 responses of these hybridomas grouped by peptide reactivity.

Altogether, we identified six apparent epitopes in the HN glycoprotein, all presented in the context of I-A^b: $HN_{163-178}$ (1 hybridoma), $HN_{205-221}$ (1 hybridoma), $HN_{415-430}$ (2 hybridomas), $HN_{421-436}$ (23 hybridomas), $HN_{475-490}$ (2 hybridomas), and $HN_{559-574}$ (5 hybridomas). All of the hybridomas that had been reactive on Vac-HN-infected LB15 cells responded to at least one of the specific HN peptides. In addition, we found that three of the five hybridomas with undetermined specificity

TABLE 3. IL-2 responses of Sendai virus HN-specific T-cell hybridomas to HN peptides presented by I-A^b stimulator cells[a]

Hybridoma	IL-2 production (U/ml)[b] by the indicated hybridoma in response to I-A^b-transfected L cells + HN peptide[c]:							
	163-178	199-215	205-221	415-430	421-436	475-490	481-496	559-574
B3.4D6	>1,280	–	–	–	–	–	–	–
B3.2A8	–	>1,280	**>1,280**	–	–	–	–	–
B3.2G9	–	–	–	>1,280	–	–	–	–
B3.1F8	–	–	–	**>1,280**	160	–	–	–
B3.2C5	–	–	–	>1,280	**>1,280**	–	–	–
B3.4A5	–	NT[d]	NT	>1,280	**>1,280**	–	–	–
B3.4F3	–	NT	NT	>1,280	**>1,280**	–	–	–
B3.3D3	–	NT	–	640	>1,280	–	–	–
B3.3E11	–	NT	–	320	**640**	–	–	–
B3.2F11	–	–	–	160	**>1,280**	–	–	–
B3.4F1	–	NT	–	160	**>1,280**	–	–	–
B3.3G5	–	NT	NT	80	640	–	–	–
B3.1F6	–	NT	NT	–	>1,280	–	–	–
B3.1G9	–	NT	NT	–	>1,280	–	–	–
B3.2F5	–	NT	–	–	>1,280	–	–	–
B3.2G4	–	NT	NT	–	>1,280	–	–	–
B3.4C2	–	–	–	–	>1,280	–	–	–
B3.4G8	–	NT	NT	–	>1,280	–	–	–
B3.3D8	–	NT	NT	–	640	–	–	–
B3.3G8	–	NT	NT	–	320	–	–	–
B3.1H4	–	NT	NT	–	320	–	–	–
B3.3E9	–	NT	NT	–	160	–	–	–
B3.3C11	–	–	–	–	80	–	–	–
B3.7H5	–	–	–	–	80	–	–	–
B3.2D4	–	–	–	–	40	–	–	–
B3.2E5	–	–	–	–	40	–	–	–
B3.5B3	–	NT	NT	–	40	–	–	–
B3.8G2	–	–	–	–	–	>1,280	–	–
B3.8D5	–	NT	–	–	–	>1,280	>1,280	–
B3.1C7	–	NT	NT	–	–	–	–	>1,280
B3.2F10	–	–	–	–	–	–	–	>1,280
B3.2H3	–	NT	NT	–	–	–	–	>1,280
B3.4G2	–	–	–	–	–	–	–	>1,280
B3.14G5	NT	NT	NT	NT	NT	NT	NT	>1,280

[a] Hybridomas are grouped according to peptide specificity. HN peptides which did not induce IL-2 secretion by any of the hybridomas are not shown.
[b] Boldface values indicate the one peptide of an overlapping pair that was identified by dose-response curves to induce the stronger IL-2 response in specific hybridomas (data not shown). –, IL-2 production below the level of detection (≤10 U/ml).
[c] Individual HN peptides were added at 30 μg/ml as described in Materials and Methods. IL-2 production by hybridomas incubated with specific peptides in the absence of presenting cells was ≤10 U/ml (data not shown).
[d] NT, not tested.

after the Vac recombinant screen (B3.2D4, B3.2E5, and B3.5B3) also responded to specific HN peptides. It is likely that presentation of HN peptides by the I-A^b transfectants is a more sensitive assay than the use of Vac-HN-infected LB15 cells.

While a majority of the hybridomas showed specificity for only one peptide, some of the hybridomas gave responses to adjacent overlapping peptides. This finding suggested that the determinant recognized by these hybridomas lay in the peptide overlap. In general, most hybridomas gave stronger responses to one peptide of an adjacent pair. For example, the response of B3.8D5 to $HN_{475-490}$ was at least 1,000-fold stronger than the response to $HN_{481-496}$ (Fig. 1). However, some hybridomas gave equivalent dose responses to adjacent peptides (B3.2A8; Fig. 1). Interestingly, the adjacent peptides $HN_{415-430}$ and $HN_{421-436}$ were each recognized preferentially by some hybridomas in dose-response assays. For example, B3.1F8 responded preferentially to $HN_{415-430}$, whereas B3.2C5 responded preferentially to $HN_{421-436}$ (Fig. 1). These data suggest that there are at least two I-A^b-restricted epitopes present in these two peptides, and we are currently trying to characterize these epitopes in more detail. The M-specific hybridoma B3.3F6 was also I-A^b restricted (data not shown), but the fine specificity of this hybridoma was not determined. B3.7F5, although reactive on Sendai virus-infected I-A^b cells, did not respond to the HN peptides (data not shown).

To test whether hybridomas with identical peptide specificities represented distinct clones, TCR Vα and Vβ usage was determined by flow cytometry and Northern (RNA) dot blot analysis. As shown in Table 4, several of the peptide epitopes were recognized by T cells expressing several distinct clonotypes. For example, at least 11 different Vα/Vβ TCR clonotypes were expressed by hybridomas specific for $HN_{421-436}$. Similarly, four of the five $HN_{559-574}$-specific hybridomas were found to use distinct Vα/Vβ combinations. These data clearly demonstrate that the predominance of HN-specific hybridomas among the hybridoma panel was not due to a preferential fusion of a limited population of T cells.

Taken together, these data show that there is a strong bias toward the HN glycoprotein in terms of MHC class II epitope selection during the course of a primary immune response to infection with Sendai virus.

DISCUSSION

This study was undertaken to define the class II-restricted epitopes recognized by $CD4^+$ T cells responding to acute

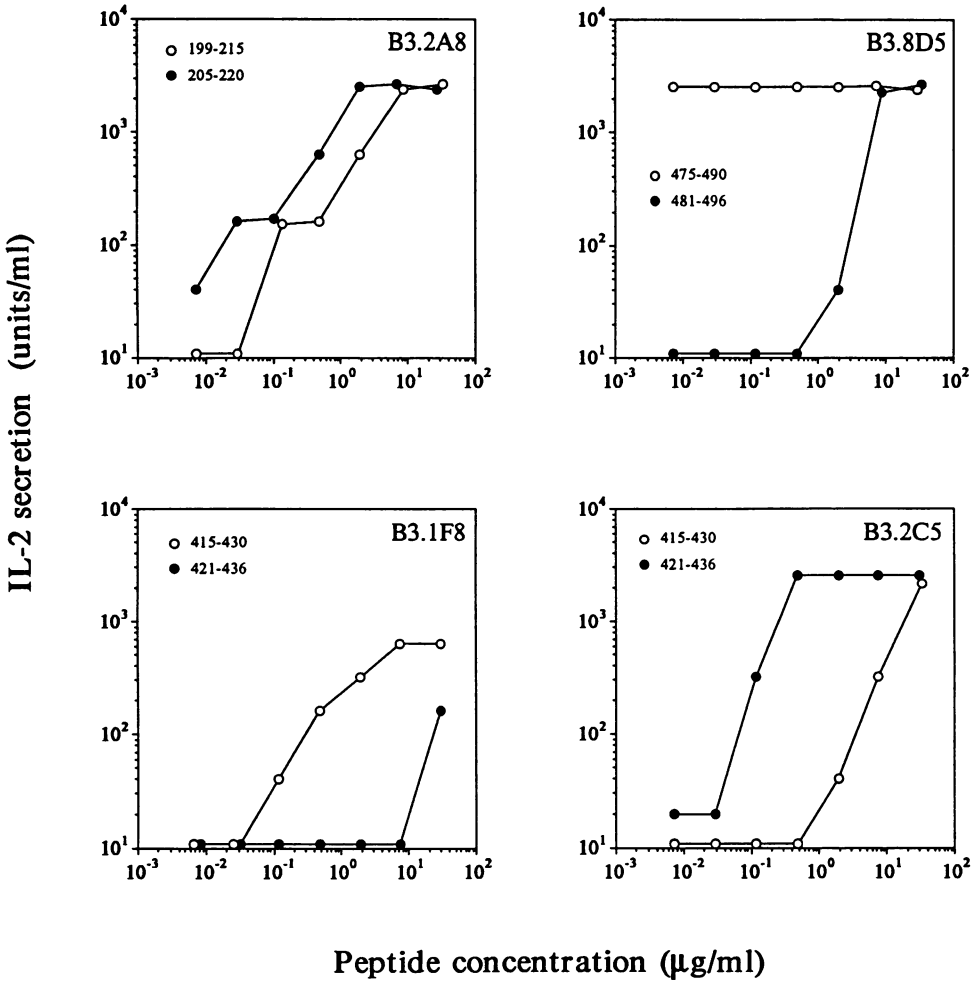

FIG. 1. Recognition of adjacent overlapping HN peptides by HN-specific hybridomas.

Sendai virus infection in B6 mice. Using hybridomas generated from the respiratory tracts of infected mice, we found that the class II-restricted Sendai virus-specific T-cell response was directed against both M and HN epitopes presented in the context of I-A^b. However, the vast majority (34 of 37) of the hybridomas were HN specific. Analysis of the HN-specific hybridomas with overlapping peptides spanning the entire HN protein identified at least five (and possibly six) I-A^b-restricted epitopes: $HN_{163-178}$, $HN_{205-221}$, possibly $HN_{415-430}$, $HN_{421-436}$, $HN_{475-490}$, and $HN_{559-574}$, with most of the hybridomas (23 out of 34) recognizing $HN_{421-436}$/I-A^b. The prominent recognition of $HN_{421-436}$/I-A^b did not reflect amplification of a single clone, since analysis of TCR Vα and Vβ usage demonstrated that this epitope was recognized by at least 11 clonotypes. Thus, these data show that the $CD4^+$ class II-restricted T-cell response to Sendai virus in B6 mice is polyclonal in terms of TCR usage. However, there is a strong bias toward epitopes derived from the HN glycoprotein.

This work extends previous studies in which cooperative interaction between $CD4^+$ and $CD8^+$ T cells has been implicated in the effective clearance of Sendai virus by B6 mice (26). While the B6 T-cell response to Sendai virus infection is mediated principally by $CD8^+$ T cells which recognize a single *H-2K*^{b}-restricted NP epitope in vitro (13, 26, 44), $CD4^+$ T cells can also mediate virus clearance and survival (21, 26). Whether immune $CD4^+$ T cells act directly in vivo to eliminate virus or provide help for some other component of the virus-specific response is not clear. Adoptive transfer studies suggest that $CD4^+$ T cells may contribute to the B6 anti-Sendai virus response by providing help to CTL in the form of IL-2 (26). Additionally, $CD4^+$ T cells are necessary in promoting antibody responses to Sendai virus (21, 26).

The amino acid sequences of the I-A^b-restricted HN peptides are shown in Table 5. Four of the six HN peptides contain the proposed I-A^b sequence motif (D)(Q)NXXXXXP(I)(S) (36). Interestingly, neither $HN_{421-436}$, which is recognized by the majority of the HN-specific hybridomas, nor $HN_{415-430}$ bears this sequence. Thus, the proposed I-A^b sequence motif may not account for all possible peptide ligands and is consistent with the fact that the MHC class II molecule structure allows for considerable flexibility in peptide binding (22, 41, 42, 48).

The most surprising aspect of this study was the strong bias toward epitopes derived from the HN glycoprotein. Thus, in a panel of 37 I-A^b-restricted $CD4^+$ hybridomas, 34 recognized HN epitopes, one recognized an M protein epitope, and the virus protein specificities of two hybridomas could not be determined. This bias was not an artifact of fusion, since multiple HN epitopes were recognized and multiple distinct TCR clonotypes were identified. The apparent bias of the

TABLE 4. TCR Vα and Vβ usage by Sendai virus-specific I-A^b-restricted T-cell hybridomas[a]

Peptide specificity	No. of hybridomas	TCR V element expressed	
		Vα	Vβ
$HN_{163-178}$	1	1	6
$HN_{205-221}$	1	6	5.1
$HN_{415-430}$	1	6	8.2
	1	6	16
$HN_{421-436}$	1	4	2
	1	4	8.1
	1	2	8.2
	1	3	8.2
	6	4	8.2
	1	4+8[b]	8.2
	4	8	8.2
	1	3	8.3
	5	4	8.3
	1	2	16
	1	11	16
$HN_{475-490}$	1	4	6
	1	4+6[b]	1
$HN_{559-574}$	1	2	3
	2	2	8.2
	1	13	8.2
	1	2	15
M	1	4	12
Sendai virus (undetermined)[c]	1	2	8.2
	1	11	8.3

[a] Hybridomas are grouped according to peptide specificity. Vα and Vβ usage by hybridomas was determined by flow cytometry using Vα/Vβ-specific MAbs and Northern dot blot analysis using a panel of Vα and Vβ DNA probes as described in Materials and Methods.
[b] Two Vα transcripts detected.
[c] Virus protein specificity could not be determined.

Sendai virus-specific CD4$^+$ T-cell response for HN recognition stands in contrast to the CD4$^+$ T-cell response to influenza virus infection. Mice infected with influenza virus mount a diverse CD4$^+$ T-cell response, with specificities for the major structural proteins represented in approximate proportions to the proteins in the virus particles (6, 23).

The difference in the CD4$^+$ T-cell recognition elicited by these two viruses may be related to the routes by which they infect cells. Sendai virus, like other members of the paramyxovirus genus, initiates infection by fusing directly with the plasma membrane of the target cell (9, 45, 46). This results in delivery of the nucleocapsid to the cytoplasm and retention of the HN and F viral envelope proteins at the infected cell plasma membrane. This may lead to preferential routing of HN and F through the class II pathway. Additionally, Sendai virus may replicate in some antigen-presenting cells. Thus, viral envelope proteins, either newly synthesized or derived directly from virion-cell membrane fusion, may become available for class II processing and presentation. Support for this possibility is found in the work of Chicz et al., who described preferential processing of integral membrane proteins through the class II pathway (12). In contrast, the influenza virion is engulfed by receptor-mediated endocytosis, and the fusion process is activated only in the low-pH environment of the late endosome (33). Thus, the endosomal localization of all influenza virus structural proteins may result in their proportional processing for association with class II molecules. One problem with this hypothesis is that Sendai virus infection should also elicit CD4$^+$ T cells for the F protein. However, none of the hybridomas described in this report were specific for this protein.

TABLE 5. Amino acid sequences of peptides recognized by I-A^b-restricted HN-specific hybridomas[a]

Peptide	Amino acid sequence
$HN_{163-178}$	VGEPYLSS**D**PKISLL**P**G
$HN_{199-215}$	SIGEAIYAYSSNLITQG
$HN_{205-221}$	YAYSSNLIT**Q**GCAD**I**GG
$HN_{415-430}$	LKLGDRVYIYTRSSGWG
$HN_{421-436}$	VYIYTRSSGWHSQLQIG
$HN_{475-490}$	SGVYT**D**AYPLS**P**DAANG
$HN_{481-496}$	AYPLSPDAANVATVTLG
$HN_{559-574}$	**Q**PMLFKT**S**IPKLCKAEG

[a] Sequences of the stimulatory peptides identified in Table 3. Putative I-A^b binding residues are shown in boldface and are assigned as specified by Rudensky et al. (41).

A second possibility is that the presence of HN-specific antibody during Sendai virus infection can bias epitope selection. This is intriguing in view of the fact that an HN MAb escape mutant has identified amino acid 420 as critical in defining HN antigenic site II (40, 50), which may overlap the I-A^b-restricted epitope(s) defined by $HN_{415-430}$ and $HN_{421-436}$. A reciprocal overlap between B-cell and CD4$^+$ T-cell epitope recognition has also been observed for influenza virus hemagglutinin, using T-cell clones isolated from the spleens of virus-primed mice (2, 3). Several groups have shown that soluble antigen-specific antibody can enhance presentation of the same antigen to T cells (7, 30, 32, 47). For example, Celis and Chang showed that antibodies specific for hepatitis B surface antigen can enhance the presentation of this antigen to T cells by 100-fold (7). This enhanced presentation was dependent on the presence of intact Fc receptors on the surface of antigen-presenting cells. In the case of Sendai virus infection, it is possible that high concentrations of HN-rich immune complexes (HN derived from the cytosolic and membrane components of lysed cells) develop in the respiratory tract at a later stage of infection. However, it follows from this proposal that the HN epitopes detected by the hybridoma panel may not be the initial specificities selected by class II restricted T cells but may arise concomitantly with the development of HN-specific antibodies.

Several observations support the idea that antibody might be influencing class II epitope selection in this system. In studying the pathogenesis of acute Sendai virus infection in the mouse, Blandford and Heath (4) showed a temporal relationship between the presence of immune complexes on the surface of desquamated respiratory epithelial cells and the detection of viral antigens in both alveolar macrophages and bronchial lymph nodes. Presence of viral antigen in alveolar macrophages is likely due to Fc receptor-mediated uptake, since these cells are not readily infected with Sendai virus in vivo (10, 34). Macrophages comprise 40 to 60% of unseparated BAL (21) and may function to nonselectively present processed viral antigens captured by immune complex uptake (53). In addition, B cells present in the BAL (19) could influence viral antigen presentation by selectively presenting membrane immunoglobulin-bound antigen (30). However, B cells are probably not involved in the primary activation of T cells (30).

Taken together, the data show that there is a bias toward the recognition of HN derived epitopes by class II-restricted CD4$^+$ T cells taken from a late stage of primary Sendai virus infection. This bias may be modulated by the nature of Sendai virus infection or by the presence of HN-specific antibody. We are currently attempting to distinguish among these possibilities experimentally.

ACKNOWLEDGMENTS

We thank Sherri Surman and Laurin Craig for excellent technical assistance, Betsy Sidell and Jim Houston for flow cytometric analyses, and Peter Doherty, Marcy Blackman, Maryna Eichelberger, and Alison Deckhut for critically reviewing the manuscript.

This work was supported by National Institutes of Health grant AI 31596 (D.L.W.) and the American Syrian Lebanese Associated Charities (ALSAC).

REFERENCES

1. **Allan, W., Z. Tabi, A. Cleary, and P. C. Doherty.** 1990. Cellular events in the lymph node and lung of mice with influenza: consequences of depleting CD8+ T cells. J. Immunol. **144:**3980–3986.
2. **Barnett, B. C., D. S. Burt, C. M. Graham, A. P. Warren, J. J. Skehel, and D. B. Thomas.** 1989. I-A^d restricted recognition of influenza hemagglutinin. Synthetic peptides identify multiple epitopes corresponding to antibody-binding regions of the HA1 subunit. J. Immunol. **143:**2663–2669.
3. **Barnett, B. C., C. M. Graham, D. S. Burt, J. J. Skehel, and D. B. Thomas.** 1989. The immune response of BALB/c mice to influenza hemagglutinin: commonality of the B cell and T cell repertoires and their relevance to antigenic drift. Eur. J. Immunol. **19:**515–521.
4. **Blandford, G., and R. B. Heath.** 1972. Studies on the immune response and pathogenesis of Sendai virus infection in mice. Immunology **22:**637–649.
5. **Braciale, T. J., and V. L. Braciale.** 1991. Antigen presentation: structural themes and functional variations. Immunol. Today **12:**124–129.
6. **Caton, A. J., and W. Gerhard.** 1992. The diversity of the CD4+ T cell response in influenza. Semin. Immunol. **4:**85–90.
7. **Celis, E., and T. W. Chang.** 1984. Antibodies to hepatitis B surface antigen potentiate the response of human T lymphocyte clones to the same antigen. Science **224:**297–299.
8. **Chakrabarti, S., K. Brechling, and B. Moss.** 1985. Vaccinia virus expression vector: coexpression of β-galactosidase provides visual screening of recombinant virus plaques. Mol. Cell. Biol. **5:**3403–3409.
9. **Chanock, R. M., and K. McIntosh.** 1990. Parainfluenza viruses, p. 963–988. *In* B. N. Fields and D. M. Knipe (ed.), Fields virology. Raven Press, New York.
10. **Charlton, D., and G. Blandford.** 1977. Immunoglobulin class-specific antibody response in serum, spleen, lungs, and bronchoalveolar lavage washings after primary and secondary Sendai virus infection of germfree mice. Infect. Imm. **17:**521–527.
11. **Cheley, S., and R. Anderson.** 1984. A reproducible microanalytical method for the detection of specific RNA sequences by dot blot hybridization. Anal. Biochem. **137:**15–19.
12. **Chicz, R. A., R. G. Urban, J. C. Gorga, D. A. A. Vignali, W. S. Lane, and J. L. Strominger.** 1993. Specificity and promiscuity among naturally processed peptides bound to HLA-DR alleles. J. Exp. Med. **178:**27–47.
13. **Cole, G. A., T. L. Hogg, and D. L. Woodland.** The MHC class I restricted T cell response to Sendai virus infection in C57BL/6 mice: a single immunodominant epitope elicits an extremely diverse repertoire of T cells. Int. Immunol., in press.
14. **Deckhut, A., W. Allan, A. McMickle, M. Eichelberger, M. A. Blackman, P. C. Doherty, and D. L. Woodland.** 1993. Prominent usage of Vβ8.3 in the H-2D^b-restricted CTL response to an influenza nucleoprotein epitope. J. Immunol. **151:**2658–2666.
15. **Dialynas, D. P., D. B. Wilde, P. Marrack, K. A. Wall, W. Havran, G. Otten, M. R. Loken, M. Pierres, J. Kappler, and F. W. Fitch.** 1983. Characterization of the murine antigenic determinant, designated L3T4a, recognized by monoclonal antibody GK1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen-reactivity. Immun. Rev. **74:**29–56.
16. **Doherty, P. C., W. Allan, and M. Eichelberger.** 1992. Role of αβ and γδ T cell subsets in viral immunity. Annu. Rev. Immunol. **10:**123–151.
17. **Endres, R. O., P. Marrack, and J. Kappler.** 1983. An IL-2-secreting T cell hybridoma that responds to a self class I histocompatibility antigen in the H-2D region. J. Immunol. **131:**1656–1666.
18. **Germain, R. N.** 1986. The ins and outs of antigen processing and presentation. Nature (London) **322:**687–689.
19. **Gorman, W. L., D. S. Gill, R. A. Scroggs, and A. Portner.** 1990. The hemagglutinin-neuraminidase glycoproteins of human parainfluenza virus type 1 and Sendai virus have high structure-function similarity with limited antigenic cross-reactivity. Virology **175:**211–221.
20. **Heilman, C.** 1990. Respiratory syncitial and parainfluenza viruses. J. Infect. Dis. **161:**402–406.
21. **Hou, S., P. C. Doherty, M. Zijlstra, and J. M. Katz.** 1992. Delayed clearance of Sendai virus in mice lacking class I MHC-restricted CD8+ T cells. J. Immunol. **149:**1319–1325.
22. **Hunt, D. F., H. Michel, T. A. Dickinson, J. Shabanowitz, A. L. Cox, K. Sakaguchi, E. Appella, H. M. Grey, and A. Sette.** 1992. Peptides presented to the immune system by the murine class II major histocompatibility complex molecule I-A^d. Science **256:**1817–1820.
23. **Hurwitz, J. L., C. J. Hackett, E. C. McAndrew, and W. Gerhard.** 1985. Murine Th response to influenza virus: recognition of hemagglutinin, neuraminidase, matrix, and nucleoprotein. J. Immunol. **134:**1994–1998.
24. **Iwai, H., K. Machu, Y. Otsuka, and K. Ueda.** 1988. T cell subsets responsible for clearance of Sendai virus from infected mouse lungs. Microbiol. Immunol. **32:**305–315.
25. **Iwai, H., S. Yamamoto, Y. Otsuka, and K. Ueda.** 1989. Cooperation between humoral factor(s) and lyt-2+ T cells in effective clearance of Sendai virus from infected mouse lungs. Microbiol. Immunol. **33:**915–925.
26. **Kast, W. M., A. M. Bronkhorst, L. P. De Wall, and C. J. Melief.** 1986. Cooperation between cytotoxic and helper T lymphocytes in protection against lethal Sendai virus infection. J. Exp. Med. **164:**723–738.
27. **Kast, W. M., L. Roux, J. Curren, H. J. J. Blom, A. C. Voordouw, R. H. Meleon, D. Kolakofsy, and C. J. M. Melief.** 1991. Protection against lethal Sendai virus infection by *in vivo* priming of virus-specific cytotoxic T lymphocytes with a free synthetic peptide. Proc. Natl. Acad. Sci. USA **88:**2283–2287.
28. **Kronenberg, M., G. Sui, L. E. Hood, and N. Shastra.** 1986. The molecular genetics of the T-cell antigen receptor and T-cell recognition. Annu. Rev. Immunol. **4:**529–591.
29. **Lamb, R. A., B. W. J. Mahy, and P. W. Choppin.** 1976. The synthesis of Sendai virus polypeptides in infected cells. Virology **69:**116–131.
30. **Lanzavecchia, A.** 1990. Receptor-mediated antigen uptake and its effect on antigen presentation to class II-restricted T lymphocytes. Annu. Rev. Immunol. **8:**773–793.
31. **Leclerq, L., G. Bismuth, and J. Thèze.** 1984. Antigen-specific helper T-cell clone supernatant is sufficient to induce both polyclonal proliferation and differentiation of small resting B lymphocytes. Proc. Natl. Acad. Sci. USA **81:**6491–6495.
32. **Manca, F., D. Fenoglio, A. Kunkl, C. Cambiaggi, M. Sasso, and F. Celada.** 1988. Differential activation of T cell clones stimulated by macrophages exposed to antigen complexed with monoclonal antibodies. J. Immunol. **140:**2893–2898.
33. **Matlin, K. S., H. Reggio, A. Helenius, and K. Simons.** 1982. Infectious entry pathway of influenza virus in a canine kidney cell line. J. Cell Biol. **91:**601–613.
34. **Mims, C. A., and F. A. Murphy.** 1973. Parainfluenza virus Sendai infection in macrophages, ependyma, choroid plexus, vascular endothelium and respiratory tract of mice. Am. J. Pathol. **70:**315–324.
35. **Morrison, L. A., A. Lukacher, V. L. Braciale, D. P. Fan, and T. J. Braciale.** 1986. Differences in antigen presentation to MHC class I- and class II-restricted influenza virus-specific cytotoxic T lymphocyte clones. J. Exp. Med. **163:**903–921.
36. **Neefjes, J. J., and H. L. Ploegh.** 1992. Intracellular transport of MHC class II molecules. Immunol. Today **13:**179–184.
37. **Panicali, D., and E. Paoletti.** 1982. Construction of poxviruses as cloning vectors: insertion of the thymidine kinase gene from herpes simplex virus into the DNA of infectious vaccinia virus. Proc. Natl. Acad. Sci. USA **79:**4927–4931.
38. **Parker, D.** 1993. T cell-dependent B cell activation. Annu. Rev.

Immunol. **11**:331–360.
39. **Portner, A., P. A. Marx, and D. W. Kingsbury.** 1974. Isolation and characterization of Sendai virus temperature-sensitive mutants. J. Virol. **13**:298–304.
40. **Portner, A., R. A. Scroggs, and D. W. Metzger.** 1987. Distinct functions of antigenic sites of the HN glycoprotein of Sendai virus. Virology **158**:61–68.
41. **Rudensky, A. Y., P. Preston-Hurlburt, B. K. Al-Ramadi, J. Rothbard, and C. A. Janeway, Jr.** 1992. Truncation variants of peptides isolated from MHC class II molecules suggest sequence motifs. Nature (London) **359**:429–431.
42. **Rudensky, A. Y., P. Preston-Hurlburt, S. Hong, A. Barlow, and C. A. Janeway, Jr.** 1991. Sequence analysis of peptides bound to MHC class II molecules. Nature (London) **353**:622–627.
43. **Sakaguchi, T., S. Takao, K. Kiyotani, Y. Fujii, T. Nakayama, and T. Yoshida.** 1993. Expression of the HN, F, NP and M proteins of Sendai virus by recombinant vaccinia viruses and their contribution to protective immunity against Sendai virus infections in mice. J. Gen. Virol. **74**:479–484.
44. **Schumacher, T. N. M., L. H. De Bruijn, L. N. Vernie, W. M. Kaste, C. J. M. Melief, J. L. Neefjes, and H. L. Ploegh.** 1991. Peptide selection by MHC class I molecules. Nature (London) **350**:703–706.
45. **Scheild, A., L. A. Caliguiri, R. W. Compans, and P. W. Choppin.** 1972. Isolation of paramyxovirus proteins: association of both hemagglutinin and neuraminidase activities with the larger SV5 glycoprotein. Virology **50**:640–652.
46. **Scheild, A., and P. W. Choppin.** 1974. Identification of biological activities of paramyxovirus glycoproteins: activation of cell fusion, hemolysis, and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. Virology **57**:475–490.
47. **Squire, C. M., E. J. Studer, A. Lees, F. D. Finkelman, and D. H. Conrad.** 1994. Antigen presentation is enhanced by targeting antigen to the FcεRII by antigen-anti-FcεRII conjugates. J. Immunol. **152**:4388–4396.
48. **Stern, L. J., J. H. Brown, T. S. Jardetzky, J. C. Gorga, R. G. Urban, J. L. Strominger, and D. C. Wiley.** 1994. Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza peptide. Nature (London) **368**:215–221.
49. **Thompson, S. D., W. G. Laver, K. G. Murti, and A. Portner.** 1988. Isolation of a biologically active soluble form of the hemagglutinin-neuraminidase protein of Sendai virus. J. Virol. **62**:4653–4660.
50. **Thompson, S. D., and A. Portner.** 1987. Localization and functional sites of the hemagglutinin-neuraminidase glycoprotein of Sendai virus by sequence analysis of antigenic and temperature-sensitive mutants. Virology **160**:1–8.
51. **Townsend, A. C. Öhlén, J. Bastin, H. G. Ljunggren, L. Foster, and K. Kärre.** 1989. Association of class I major histocompatibility heavy and light chains induced by viral peptides. Nature (London) **340**:443–448.
52. **Townsend, A. R., J. Rothbard, F. M. Gotch, G. Bahadur, D. Wraith, and A. J. McMichael.** 1986. The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. Cell **44**:959–968.
53. **Unanue, E. R., and P. M. Allen.** 1987. The basis for the immunoregulatory role of macrophages and other accessory cells. Science **236**:551–557.
54. **White, J., M. Blackman, J. Bill, J. Kappler, P. Marrack, D. P. Gold, and W. Born.** 1989. Two better cell lines for making hybridomas expressing specific T cell receptors. J. Immunol. **143**: 822–825.
55. **Woodland, D. L., M. P. Happ, J. Bill, and E. Palmer.** 1990. Requirement for cotolerogenic gene products in the clonal deletion of I-E reactive cells. Science **247**:964–967.