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Enumeration of Antigen-Presenting Cells in Mice Infected with Sendai Virus[1]

Edward J. Usherwood,* Twala L. Hogg,* and David L. Woodland[2]*†

Substantial progress has been made in understanding Ag presentation to T cells; however, relatively little is known about the location and frequency of cells presenting viral Ags during a viral infection. Here, we took advantage of a highly sensitive system using *lacZ*-inducible T cell hybridomas to enumerate APCs during the course of respiratory Sendai virus infection in mice. Using *lacZ*-inducible T cell hybridomas specific for the immunodominant hemagglutinin-neuraminidase $HN_{421-436}$/I-A^b and nucleoprotein $NP_{324-332}$/K^b epitopes, we detected APCs in draining mediastinal lymph nodes (MLNs), in cervical lymph nodes, and also in the spleen. $HN_{421-436}$/I-A^b- and $NP_{324-332}$/K^b-presenting cells were readily detectable between days 3 and 9 postinfection, with more APCs present in the MLN than in the cervical lymph nodes. Interestingly, no infectious virus was detected in lymphoid tissue beyond day 6, suggesting that a depot of noninfectious viral Ag survives, in some form, for 2–3 days after viral clearance. Fractionation of the MLN demonstrated that APC frequency was enriched in dendritic cells and macrophages but depleted in the B cell population, suggesting that B cells do not form a large population of APCs during the primary response to this virus. ***The Journal of Immunology*, 1999, 162: 3350–3355.**

Activation of naive T lymphocytes requires an interaction with professional APCs expressing appropriate MHC/peptide complexes plus the correct costimulatory and adhesion molecules on the cell surface. It is believed that APCs acquire viral Ags at the site of infection and migrate to the local lymphoid tissue, where they activate resting T cells. In respiratory infections such as that caused by Sendai virus (a murine parainfluenza type 1 virus), viral proteins are acquired by APCs in the lung, which then migrate to the mediastinal lymph nodes (MLNs)[3] and cervical lymph nodes (CLNs). These cells present viral Ag to the naive Ag-specific T cells in the lymph nodes, which subsequently become activated and traffic to the lung. This model is supported by data showing that Sendai virus-specific T cells are detectable using limiting dilution analysis in the MLN at day 5 postinfection; however, these cells do not appear in the bronchoalveolar lavage (BAL) until day 8 postinfection (1). More recent work with tetrameric MHC/peptide reagents has shown that >70% of the $CD8^+$ T cells in the BAL are specific for the dominant epitope during acute infection (day 10). In addition, ~6% of $CD8^+$ T cells in the spleens of mice that have recovered from infection are specific for the immunodominant epitope (E.J.U. and D.L.W., manuscript in preparation).

The frequency and kinetics of Ag presentation in viral systems have not been extensively studied. Sendai virus is pneumotropic, infecting only ciliated and secretory cells in the trachea and bronchi and type II epithelial cells in the lung (2, 3). Presentation of viral Ags on professional APCs occurs when these cells are infected with the virus or following phagocytosis of infected lung cells or virions. The lung has a contiguous network of MHC class II-positive dendritic cells (DCs) that turn over rapidly and may constantly sample the antigenic environment in the lungs and transport Ag to draining lymph nodes (4). These cells increase in number during Sendai virus infection in rats and remain elevated for ≥14 days after infection (5). Work by Hamilton-Easton and Eichelberger (6) in the influenza virus system has shown that T cell hybridomas can be used to detect viral Ag on APCs after viral infection. These studies showed that cells presenting viral epitopes were present at 2 days postinfection; however, the numbers of APCs could not be quantitated accurately. In the current report, we took advantage of a new technology for generating *lacZ*-inducible T cell hybridomas, which are significantly more sensitive than conventional hybridomas (7, 8), to quantitate the numbers of cells presenting viral Ag during the first 10 days after Sendai virus infection of C57BL/6 mice. The data show that APCs are readily detectable and persist for several days after the virus has been cleared from draining lymph nodes, suggesting that viral Ag can persist in a noninfectious form for ≥2–3 days.

Materials and Methods

Mice and virus

Female C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed under specific pathogen-free conditions. Mice were infected at 6–12 wk of age. The Enders strain of Sendai virus and the A/HK-x31 (H3N2) strain of influenza virus were grown, stored, and titrated as described previously (9, 10). Mice were anesthetized by i.p. injection with Avertin (2,2,2-tribromoethanol) and infected intranasally with 500 50% egg infectious doses of Sendai virus or 240 hemagglutination units of influenza virus.

Abs and cell sorting

All of the Abs used for staining were purchased from PharMingen (San Diego, CA). MLNs or spleen cells were stained for three-color cell sorting with combinations of the following Abs: anti-CD45R phycoerythrin (PE)

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[3] Abbreviations used in this paper: MLN, mediastinal lymph node; CLN, cervical lymph node; BAL, bronchoalveolar lavage; DC, dendritic cell; PE, phycoerythrin; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside; HN, hemagglutinin-neuraminidase; NP, nucleoprotein.

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conjugate (B220; cat no. 01125B), anti-CD45R Cy5 conjugate (B220; cat no. 01128B), anti-CD11c FITC conjugate (09704D), CD11c PE conjugate (09705B), anti-CD11b biotin conjugate (01712D), CD11b PE conjugate (01715B), and streptavidin Cy-Chrome (13038A). Samples were sorted on a FACStar^{Plus} flow cytometer (Becton Dickinson, San José, CA). Four populations of cells were collected: $CD11c^+CD45R^-$ (DCs), $CD11b^+CD45R^-$ (macrophages), $CD45R^+$ (B cells), and $CD11c^-CD11b^-CD45R^-$ cells. The yields of cells were as follows: $CD11c^+CD45R^-$ cells, 1.5–2% in MLNs and spleen; $CD11b^+CD45R^-$, 6–7% from spleen and 1.5–2% from MLNs; $CD45R^+$ cells, 50–60%. In general, the purity after sorting was ≥90%.

Cell lines and culture conditions

All cell lines were grown in complete tumor medium containing 10% FCS at 37°C with 10% CO_2 (11). L cells transected with the K^b or I-A^b MHC genes have been described previously (12, 13). The BWZ.36 fusion partner (8, 14) was a gift of Dr. Nilabh Shastri (University of California, Berkeley, CA).

Synthetic peptides

Sendai virus nucleoprotein $NP_{324–332}$ (FAPGNYPAL) and hemagglutinin-neuraminidase $HN_{421–436}$ (VYIYTRSSGWHSQLQIG) peptides were synthesized at St. Jude Children's Research Hospital Center for Biotechnology on an Applied Biosystems model 433A peptide synthesizer (Applied Biosystems, Berkeley, CA). Peptide purity was evaluated using reverse-phase HPLC analysis. Stock solutions of peptides (1 mg/ml) were made in PBS and subsequently diluted in complete tumor medium before use in assays.

LacZ-inducible T cell hybridomas

MLNs were removed from C57BL/6 mice at 10 days after intranasal infection with 500 50% egg infectious doses of Sendai virus, and cell suspensions were cultured with 10 U/ml human rIL-2 for 2 days. Live cells were separated by centrifugation into lymphocyte separation medium (ICN Biomedicals, Aurora, OH) and cultured overnight in fresh medium with 10 U/ml human rIL-2. Blast cells were subsequently harvested and fused with BWZ.36 cells, which contain the *lacZ* gene fused to the NF of activated T cells-activated enhancer from the IL-2 gene. After selection for peptide specificity, each line was subcloned using the autocloning facility on a FACStar^{Plus} flow cytometer (Becton Dickinson). Subclones were screened using L cells transfected with either K^b or I-A^b molecules plus either peptide $NP_{324–332}$ (K^b-restricted hybridomas) or peptide $HN_{421–436}$ (I-A^b-restricted hybridomas) at 10 μg/ml. Those clones that produced ~50% or more $lacZ^+$ cells in response to peptide stimulation were selected for further study.

APC assays

APCs were added to flat-bottom, 96-well plates (Sarstedt, Newton, NC) plus peptide where appropriate. A total of 10^5 hybridoma cells were added per well, the cultures were incubated overnight, and a 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) assay was performed the following morning. Experiments with L-K^b or L-I-A^b cells used 10^5 L cells/well. For peptide loading, L cells were incubated with 50 μg/ml peptide at 37°C for 4 h and then washed four times. In some experiments, cells were infected with Sendai virus at 37°C for 1 h at a multiplicity of 10 followed by four washes. For an ex vivo estimation of APC frequency, CLNs, MLNs, and spleens were removed from groups of four to six mice at various times postinfection; next, the organs were pooled, and single-cell suspensions were prepared. Twofold serial dilutions of cells from each organ were made in flat-bottom, 96-well plates starting at 10^6 cells/well. A total of 10^5 hybridoma cells were added to each well and cultured overnight; next, an X-Gal assay was performed to identify responding hybridomas. The number of cells in the highest dilution of APCs giving twice the background number of responding hybridomas was taken as the reciprocal frequency. To assess background *lacZ* expression, hybridomas were cultured with lymph node or spleen cells from mice infected with influenza virus. A *lacZ*-inducible hybridoma specific for glycoprotein B of murine gammaherpesvirus 68 (4951.5) was used as a further negative control (L. Liu, E. Flano, E.J.U. S. Surman, M. A. Blackman, and D.L.W., unpublished data). This hybridoma was not stimulated in response to APCs presenting Sendai virus epitopes. As a positive control, hybridomas were cultured with spleen or lymph node cells in the presence of 10 μg/ml of the relevant peptide.

X-Gal assay

Cultures in flat-bottom 96-well plates were washed with 200 μl of PBS per well and subsequently fixed by the addition of 100 μl of PBS containing 2% formaldehyde and 0.2% glutaraldehyde for 5 min at 4°C. The plates were washed with PBS and then overlaid with 50 μl of a solution containing 1 mg/ml X-Gal, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, and 2 mM $MgCl_2$ in PBS. Cultures were examined microscopically, and the number of blue cells was counted after 6–8 h of incubation at 37°C or after incubation overnight at 4°C.

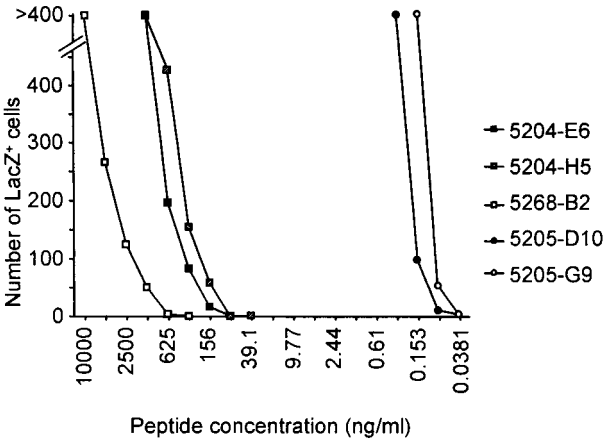

FIGURE 1. Sensitivity of *lacZ*-inducible hybridomas to peptide. Subclones derived from the hybridomas 5204 and 5268 ($HN_{421–436}$/I-A^b-specific; squares) or 5205 ($NP_{324–332}$/K^b-specific; circles) were cultured with L-I-A^b or L-K^b cells plus a graded concentration of the relevant peptide.

Detection of virus in lymphoid tissue

Cell suspensions prepared from lymph nodes and spleen were directly injected into 10-day-old embryonated hen eggs. After incubation at 35°C for 48 h, allantoic fluid from each egg was sampled and assayed for hemagglutinating activity using 0.5% chicken E in a volume of 50 μl. Organs from four mice were pooled at each timepoint, and 10^7 cells were injected into three eggs each. Samples were scored as positive when at least two of the three eggs contained hemagglutinating activity.

Results

Derivation and selection of lacZ-inducible T cell hybridomas

To investigate Ag presentation in Sendai virus-infected mice, we first generated *lacZ*-inducible T cell hybridomas specific for the immunodominant MHC class I- and class II-restricted epitopes. MLNs were harvested from C57BL/6 mice at 10 days after Sendai virus infection and cultured in vitro for an additional 3 days in the presence of IL-2; next, T cell blasts were fused with the BWZ.36 cell line. A total of 71 hybridomas were generated, two of which recognized the immunodominant $NP_{324–332}$/K^b epitope and five of which recognized the dominant $HN_{421–436}$/I-A^b epitope. Subclones were derived from these hybridomas, and those that gave the strongest response to peptide (in terms of the percentage of $lacZ^+$ cells) were selected. We subsequently tested the sensitivity of these hybridoma subclones to graded doses of peptide (Fig. 1). The I-A^b-restricted hybridomas (5204-E6, 5204-H5, and 5268-B2) responded to peptide concentrations as low as 1.25 μg/ml to 156 ng/ml, whereas both of the K^b-restricted hybridomas (5205-D10 and 5205-G9) responded to concentrations in the 153–76 pg/ml range. The subclones 5204-H5 ($HN_{421–436}$/I-A^b-restricted) and 5205-G9 ($NP_{324–332}$/K^b-restricted) were the most sensitive and consequently were chosen for further study.

It has been established that *lacZ*-inducible hybridomas are sensitive to lower APC numbers than conventional hybridomas (8). To establish the sensitivity of our Sendai virus-specific hybridomas, we assessed their ability to respond to low numbers of L-K^b or L-I-A^b cells that had been either infected with Sendai virus or loaded with the relevant peptide. As shown in Fig. 2, both hybridomas responded to smaller numbers of peptide-loaded APCs than

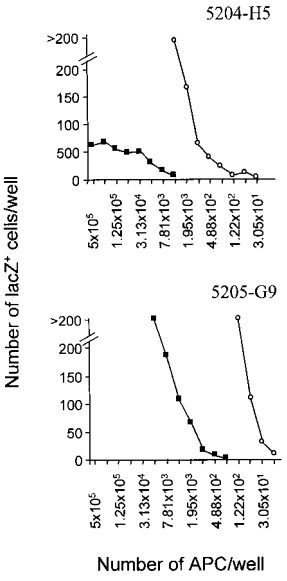

FIGURE 2. Sensitivity of Sendai virus-specific T cell hybridomas to APC number. Graded numbers of L-K^b or L-I-A^b cells were either infected with Sendai virus (■) or loaded with $NP_{324-332}$ or $HN_{421-436}$ peptide, respectively (○), and then used as APCs. Background was assessed by culturing the hybridomas with the appropriate untreated L cells. Hybridoma 5205-G9 ($NP_{324-332}/K^b$-specific) had very low background numbers of $lacZ^+$ cells (usually <5 cells/well), whereas the background with hybridoma 5204-H5 ($HN_{421-436}$/I-A^b-specific) was more variable and generally higher (typically 20–30 cells/well).

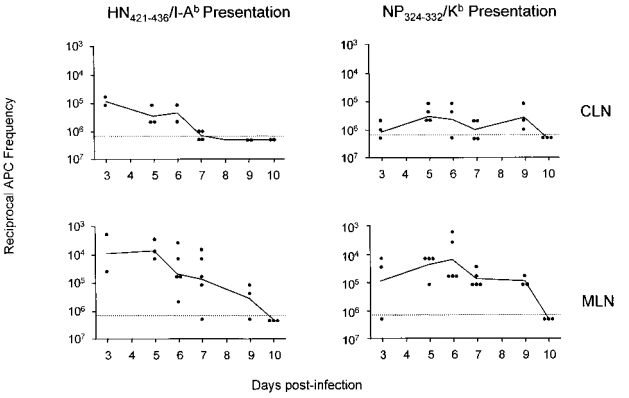

FIGURE 3. Frequencies of cells presenting the immunodominant MHC class I- and MHC class II-restricted epitopes in CLNs and MLNs following Sendai virus infection. Hybridomas 5204-H5 ($HN_{421-436}$/I-A^b-specific) or 5205-G9 ($NP_{324-332}/K^b$-specific) were cultured with graded numbers of CLN or MLN cells (pooled from four to six mice). The number of cells in the highest dilution of cells that gave twice the background number of $lacZ^+$ cells was taken to be the reciprocal frequency. Background was measured by culture of the hybridomas with the same organs taken from influenza virus-infected mice. Background was similar regardless of the number of influenza virus-infected APCs (typically <5 cells/well for 5205-G9 and 20–30 cells/well for 5204-H5). The points on the graphs represent the frequencies obtained in different experiments, and the line connects the geometric mean of all frequencies at a given timepoint. Points below the dotted line were below the limit of detection of the assay.

virus-infected APCs. This difference probably reflects a higher density of peptide-MHC complexes after peptide loading. The $NP_{324-332}/K^b$-restricted 5205-G9 hybridoma required ~30 peptide-loaded cells or ~2000 virus-infected cells to give a response above twice the background (untreated APCs). The $HN_{421-436}$/I-A^b-restricted 5204-H5 hybridoma required ~250 peptide-loaded cells or ~8700-virus infected cells. Experiments using whole spleen cells as APCs yielded similar results (data not shown). These data indicate that Sendai virus-specific *lacZ*-inducible hybridomas can detect very low numbers of APCs presenting viral epitopes and could potentially be used to detect APCs from virus-infected mice.

Measurement of APC frequencies in lymphoid organs from infected mice

Little is known about the frequency of cells presenting particular viral epitopes during viral infection, so we designed an experiment to determine 1) whether APCs were present at a sufficient level to be detectable and 2) how long Ag presentation lasted after the clearance of virus. We infected C57BL/6 mice with Sendai virus, and then removed CLNs, MLNs, and spleens at days 3–10 postinfection. Graded numbers of cells from these organs were incubated with the lacZ-inducible 5205-G9 and 5204-H5 hybridomas. As a negative control, C57BL/6 mice were infected with influenza x31 virus; at each timepoint, the lymph nodes and spleens of these control mice were titrated in the same way as for Sendai virus-infected mice. This gave an estimate of the background level of activation of the hybridomas in response to APCs from mice during an immune response to an unrelated virus.

Due to the small size of the MLN in the first 2 days after infection, our study began at day 3 postinfection. At this time, we could detect relatively high frequencies of cells in the MLN presenting viral Ags (1:9 × 10^3 cells presenting $HN_{421-436}$/I-A^b and 1:9.7 × 10^4 cells presenting $NP_{324-332}/K^b$) (Fig. 3). APCs presenting the $NP_{324-332}/K^b$ epitope in the MLN remained at a frequency of ≥1:10^5 cells until day 10, when the frequency fell to <1 in 10^6. $HN_{421-436}$/I-A^b presentation in the MLN began at a frequency of ~1:10^4 cells at days 3–5 and then steadily declined to undetectable levels by day 10. In the CLN, the pattern was similar, except APC frequencies were 10- to 20-fold lower. Detection of APCs in the spleen was variable, and the frequency was at the limit of detection of these assays (~1:10^6) when unfractionated cells were used. These figures provided a minimal estimate of the frequency of APCs, as none of the hybrids appeared to recognize <30 cells/well, and APCs expressing a low density of Ag were probably not detectable. It should be noted that the data in Fig. 3 indicate that there is a two-log variation in the titers of APCs presenting $NP_{324-332}/K^b$ or $HN_{421-436}/A^b$ epitopes. Given the fact that viral titers can vary by several logs in the lungs of mice infected with the same dose of virus, these data probably reflect the inherent variation between individual infections. Experiments under controlled conditions using exogenously added peptides indicate that the hybridoma assay of APC frequencies is very consistent and usually only shows variation within one to two doubling dilutions.

To control for the increase in cellularity of the draining lymph nodes during infection, we calculated an estimate of the actual number of detectable APCs present in each organ (Fig. 4). When presented in this way, the data reveal a large increase in APC numbers between days 3 and 5, corresponding to the dramatic increase in size of the MLN. This was not the case for the $HN_{421-436}$/I-A^b response in the CLN, where the numbers of APCs remained approximately the same between days 3 and 6. At the peak, the number of $HN_{421-436}$/I-A^b-presenting cells was approximately threefold higher than $HN_{324-332}/K^b$-presenting cells in the MLN (note different scales on Fig. 4), whereas these numbers were more similar in the CLN.

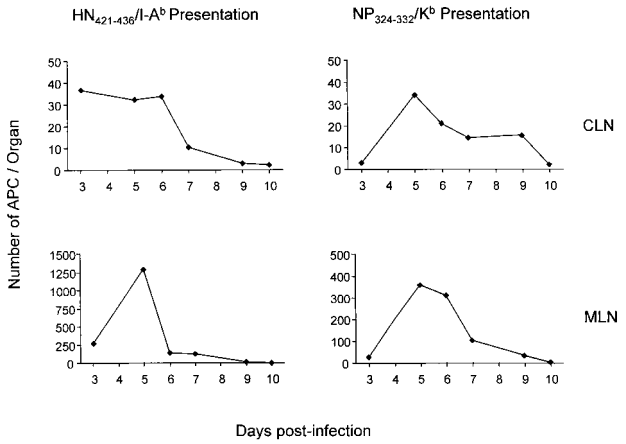

FIGURE 4. Numbers of cells presenting Sendai virus epitopes in draining lymph nodes from infected mice. The geometric means of the frequency of APCs were taken from the data presented in Fig. 3 and multiplied by the mean numbers of cells present in the appropriate lymph nodes at each timepoint. This gives a minimum estimate of the number of cells presenting viral epitopes in the CLNs and MLNs.

Ag presentation in the lymph nodes could be the result of a constant traffic of live virus from the lung to the local lymph nodes. Alternatively, there could be a depot of Ag at this site that was constantly "sampled" by APCs. To determine whether infectious virus was present in lymphoid tissue, we sampled these tissues at various times after infection, inoculated cells into embryonated hen eggs, and performed hemagglutination assays (Table I). Virus was not detected at any timepoint studied in the CLN or spleen but was detectable in the MLN at days 3 and 6 postinfection. This finding is consistent with previous reports (15). Because cells presenting both MHC class I and MHC class II epitopes were present in the absence of detectable virus (CLN and MLN after day 6), the data indicate that viral Ag is persisting in some form in these tissues. Clearly, more experiments are needed to determine the mechanism and form of Ag persistence.

Phenotype of APCs

As we could readily detect APCs ex vivo, we subsequently asked what are the major cell types presenting viral epitopes in the primary response? We used FACS to fractionate MLNs or spleen cells from infected mice into enriched populations of B cells ($CD45R^+$), DCs ($CD45R^-CD11c^+$), or macrophages ($CD45R^-CD11b^+$). Day 6 was chosen for these studies, as this was a time when a high frequency of APCs was detectable in unfractionated lymph nodes. As shown in Table II, we observed a large enrichment in the frequency of $HN_{421-436}$/I-A^b- and $NP_{324-332}$/K^b-presenting cells in the macrophage and DC fractions. In contrast, the B cell fraction was depleted of I-A^b- and K^b-presenting cells. This observation that very few B cells were presenting viral epitopes during the infection was surprising in view of the vigorous B cell response to the virus (16). The major APCs at 6 days postinfection were DCs and macrophages.

Interestingly, $HN_{421-436}$/I-A^b-presenting cells could also be detected in the spleen in the macrophage and DC fractions, albeit at very low levels. This low frequency of APCs explains why we could only detect APCs in the spleen sporadically using unfractionated spleen cells. Nonetheless, these data clearly demonstrate that viral Ag-coated APCs seed the spleen in addition to the local lymph nodes during the first 6 days of infection.

Table I. *Detection of virus in lymphoid organs following infection of C57BL/6 mice with Sendai virus*

	Days Postinfection[a]					
	3	6	7	8	9	10
CLN	−	−	−	−	−	−
MLN	+	+	−	−	−	−
Spleen	+/−	−	−	−	−	−

[a] C57BL/6 mice were infected with Sendai virus; next, the CLN, MLN, and spleens were removed at the appropriate times postinfection. Organs from four mice were pooled for the day 3 and day 6 timepoints, and organs from three individual mice were prepared separately for later timepoints. Cell preparations were injected into embryonated hen eggs as described in *Materials and Methods*. + represents detection of hemagglutinating activity in at least two of three eggs tested; +/− represents weak hemagglutinating activity in at least two of three eggs; − represents hemagglutinating activity in less than two of three eggs.

Discussion

Many studies have focused on the molecular and biochemical aspects of MHC class I- and class II-restricted Ag presentation to the immune system. However, relatively little attention has been paid to Ag presentation in vivo in the context of a pathogenic infection. In the current manuscript, we have taken advantage of *lacZ*-inducible T cell hybridomas to study the kinetics of Ag presentation in mice following infection with Sendai virus. The data show that 1) APCs presenting immunodominant MHC class I- and MHC class II-restricted epitopes were detectable between days 3 and 9 in the MLN, 2) Ag presentation continued for several days after clearance of infectious virus, and 3) Ag presentation also occurred in the spleen in the absence of infectious virus. These observations extend our understanding of Ag presentation in the context of a live virus infection.

LacZ-inducible T cell hybridomas have been used successfully to identify new minor histocompatibility Ags by expression cloning (17) and also to study the cells presenting Ag after DNA vaccination (18). The hybridomas were found to have responses that were identical with those of conventional hybridomas, and the peptide sensitivity and signal to noise ratio was comparable (8). Because of the ability to detect activation in single cells, the lacZ-inducible hybridomas were 50- to 100-fold more sensitive than conventional hybridomas at detecting small numbers of APCs. Our T cell hybridoma specific for $NP_{324-332}$/K^b responded to 30 peptide-loaded APCs or 2000 virus-infected cells/well, and the $HN_{421-436}$/I-A^b hybridoma recognized 250 peptide-loaded or 8700 virus-infected APCs. These figures were established using nonprofessional APCs (L cells), and it is possible that the detection limit may be lower using the professional APCs located in lymphoid organs. Therefore, the APC frequencies obtained in this study should be taken as a minimum estimate. Despite this limitation, APCs were easily detectable ex vivo and could be detected several days after the clearance of infectious virus from lymphoid tissue. An alternative approach to quantifying Ag/MHC complexes has been described by Dadaglio et al. and Porgador et al. using Abs against specific peptide/MHC complexes (19, 20). The Ab approach has an advantage in that it allows direct quantification of peptide/MHC complexes on the cell surface and can be used in situ. However, it has a disadvantage in that it is difficult to generate and confirm the specificity of Ab reagents, particularly if reagents for several epitopes are required. It is not clear how the hybridoma and Ab techniques compare with each other in terms of sensitivity, although both approaches are not likely to detect low levels of peptide/APC complexes.

The number of APCs reached a peak in lymph nodes at 5 days postinfection, before the peak CTL response, which is consistent

Table II. *Fractionation of APCs from lymphoid organs during acute Sendai virus infection*

Cell Fraction[a]	Unsorted	$CD45^-CD11b^-CD11c^-$	$CD45R^+$	$CD11b^+CD45R^-$	$CD11c^+CD45R^-$
MLN[b,c]					
5204 H5	6×10^4	3.1×10^4 (1.94[d])	ND	2.8×10^3 (21.4)	2.3×10^2 (261)
	1.25×10^5	ND	$>1.25 \times 10^6$ (<0.1)	5.75×10^3 (21.7)	8.13×10^3 (15.3)
5205 G9	6×10^4	3.25×10^5 (0.2)	ND	4.25×10^3 (14.1)	1.55×10^3 (38.7)
	5×10^5	ND	$>1.25 \times 10^6$ (<0.1)	$>1.15 \times 10^4$ (<43)	8.13×10^3 (61.5)
Spleen					
5204 H5	10^6	$>10^6$	ND	6×10^5 (1.7)	1.25×10^5 (4)
	$>10^6$	ND	$>1.25 \times 10^6$ (<0.8)	$>1.5 \times 10^5$ (<6.7)	4.5×10^5 (>2.2)
5205 G9	$>10^6$	$>10^6$	ND	$>6 \times 10^5$	$>2.5 \times 10^5$
	$>10^6$	ND	$>1.25 \times 10^6$	$>1.25 \times 10^5$	$>9 \times 10^5$

[a] Cells from MLNs or spleen were stained with anti-CD45R (B220) Cy5, anti-CD11b PE and, anti-CD11c FITC Abs and the following populations were collected by FACS: $CD45R^+$ cells (B cells), $CD11b^+CD45R^-$ (macrophages), and $CD11c^+CD45R^-$ cells (DCs).

[b] Reciprocal cell frequencies are presented. Where reciprocal frequencies are expressed as greater than a certain value, this means that no significant response was detected from the lacZ-inducible hybrids at the maximum number of cells per well of this APC type. The cell frequency was calculated from the minimum number of APCs that produced at least twice the number of $lacZ^+$ cells that were observed in control wells (those containing APCs from influenza-infected mice).

[c] Two rows are shown for each hybrid, representing duplicate experiments selected from a total of three to four.

[d] Numbers in parentheses refer to the increase in frequency relative to the unsorted population. Thus, numbers <1 represent a depletion in the APC frequency, and numbers >1 represent an enrichment.

with the fact that naive CTLs require activation by APCs in the draining lymph node before they can migrate to the lung as differentiated effector CTLs. At this time after infection, viable virus can be detected in the MLN using a sensitive egg infection assay. However, no detectable virus is present after 6 days postinfection in the draining lymph node, but $NP_{324-332}/K^b$- and $HN_{421-436}/I\text{-}A^b$-presenting cells are still detectable up to day 9 postinfection. It is unlikely that the *lacZ*-inducible hybridoma assay is more sensitive than the egg infection assay for detecting infectious virus. This implies that viral Ags persist in a noninfectious form for several days. There are several possible sources for this persistent Ag. Viral proteins produced within infected APCs may be degraded slowly, so that peptide epitopes are generated over several days until the supply is exhausted. Alternatively, there may be an extracellular depot of Ag that is constantly sampled by DCs which are able to present nonreplicating virus particles on MHC class I molecules (21) in addition to MHC class II molecules. Yet another possibility is that there may be limited transcription from persisting viral genomes.

It has been assumed that in respiratory infections Ag presentation occurs exclusively in the draining lymph nodes and that specific T cells only migrate to the spleen following activation. In fact, we observed APCs in the spleen, albeit at very low levels. This observation would imply that antiviral T cells can be activated directly at this site, rather than the spleen being merely a repository for T cells that were activated elsewhere. This would explain recent data in the influenza virus system showing a low but detectable level of specific CTLs in the spleen at early times after infection (0.1–0.5% of $CD8^+$ T cells at 7 days postinfection) (22).

Fractionation of the APC population revealed a large enrichment for cells presenting viral epitopes in the macrophage and DC populations, which is consistent with data obtained in influenza infection using conventional hybridoma cells (6). In contrast, APC frequency was markedly depleted in the B cell fraction, suggesting that B lymphocytes are not a major APC population in the primary Sendai virus infection. In the influenza virus study, B lymphocytes taken from the BAL were shown to present Ag. However, this may be due to the fact that B cells in the lung are exposed to a very large amount of Ag, so that even inefficient Ag presentation would be sufficient to stimulate T cell hybridomas. Ag presentation locally in the draining lymph node is likely to be of more importance physiologically, as it is here, rather than in the lung, where naive T cells become activated. It would be interesting to test whether B cells are more important APCs in a secondary response, because there would be an elevated number of Sendai virus-specific B cells expressing high affinity Ab, which is known to enhance the APC function of B cells (23).

In conclusion, new and sensitive techniques now allow us to detect relatively low frequencies of APCs ex vivo. In this study, we used an infection where the virus is completely cleared in a short period of time; however, the system is broadly applicable, and it would be interesting to apply this technique to a persistent infection. Study of the kinetics of Ag presentation is important, because efficient Ag presentation is a general requirement for T cell-mediated immune responses. Understanding this vital step may lead to a better understanding of the processes that interrupt the generation of an efficient immune response and may provide insight for the development of more rational vaccines.

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References

1. Hou, S., and P. C. Doherty. 1995. Clearance of Sendai virus by $CD8^+$ T cells requires direct targeting to virus-infected epithelium. *Eur. J. Immunol. 25:111.*
2. Massion, P. P., C. C. Funari, I. Ueki, S. Ikeda, D. M. McDonald, and J. A. Nadel. 1993. Parainfluenza (Sendai) virus infects ciliated cells and secretory cells but not basal cells of rat tracheal epithelium. *Am. J. Respir. Cell Mol. Biol. 9:361.*
3. Castleman, W. L., P. J. Northrop, and P. K. McAllister. 1989. Replication of parainfluenza (Sendai) virus in isolated rat pulmonary type II alveolar epithelial cells. *Am. J. Pathol. 134:1135.*
4. Holt, P. G., S. Haining, D. J. Nelson, and J. D. Sedgwick. 1994. Origin and steady-state turnover of class II MHC-bearing dendritic cells in the epithelium of the conducting airways. *J. Immunol. 153:256.*
5. McWilliam, A. S., A. M. Marsh, and P. G. Holt. 1997. Inflammatory infiltration of the upper airway epithelium during Sendai virus infection: involvement of epithelial dendritic cells. *J. Virol. 71:226.*
6. Hamilton-Easton, A., and M. Eichelberger. 1995. Virus-specific antigen presentation by different subsets of cells from lung and mediastinal lymph node tissues of influenza virus-infected mice. *J. Virol.69:6359.*
7. Karttunen, J., S. Sanderson, and N. Shastri. 1992. Detection of rare antigen-presenting cells by the lacZ T-cell activation assay suggests an expression cloning strategy for T-cell antigens. *Proc. Natl. Acad. Sci. USA 89:6020.*
8. Sanderson, S., and N. Shastri. 1994. LacZ-inducible, antigen/MHC-specific T cell hybrids. *Int. Immunol. 6:369.*
9. Hou, S., J. M. Katz, P. C. Doherty, and S. R. Carding. 1992. Extent of γδ T cell involvement in the pneumonia caused by Sendai virus. *Cell. Immunol. 143:183.*
10. Daly, K., P. Nguyen, D. L. Woodland, and M. A. Blackman. 1995. Immunodominance of major histocompatibility complex class I-restricted influenza virus epitopes can be influenced by the T-cell receptor repertoire. *J. Virol. 69:7416.*

11. Cole, G. A., T. L. Hogg, M. A. Coppola, and D. L. Woodland. 1997. Efficient priming of $CD8^+$ memory T cells specific for a subdominant epitope following Sendai virus infection. *J. Immunol. 158:4301.*
12. Cole, G. A., V. K. Clements, E. P. Garcia, and S. Ostrand-Rosenberg. 1987. Allogeneic H-2 antigen expression is insufficient for tumor rejection. *Proc. Natl. Acad. Sci. USA 84:8613.*
13. Ostrand-Rosenberg, S., C. Roby, V. K. Clements, and G. A. Cole. 1991. Tumor-specific immunity can be enhanced by transfection of tumor cells with syngeneic MHC-class-II genes or allogeneic MHC-class-I genes. *Int. J. Cancer Suppl. 6:61.*
14. Shastri, N. 1995. Single T cell probes for antigen/MHC expression. *Curr. Opin. Immunol. 7:258.*
15. Hou, S., P. C. Doherty, M. Zijlstra, R. Jaenisch, and J. M. Katz. 1992. Delayed clearance of Sendai virus in mice lacking class I MHC-restricted $CD8^+$ T cells. *J. Immunol. 149:1319.*
16. Mo, X. Y., M. Sangster, S. Sarawar, C. Coleclough, and P. C. Doherty. 1995. Differential antigen burden modulates the γ interferon but not the immunoglobulin response in mice that vary in susceptibility to Sendai virus pneumonia. *J. Virol. 69:5592.*
17. Mendoza, L. M., P. Paz, A. Zuberi, G. Christianson, D. Roopenian, and N. Shastri. 1997. Minors held by majors: the H13 minor histocompatibility locus defined as a peptide/MHC class I complex. *Immunity 7:461.*
18. Casares, S., K. Inaba, T. D. Brumeanu, R. M. Steinman, and C. A. Bona. 1997. Antigen presentation by dendritic cells after immunization with DNA encoding a major histocompatibility complex class II-restricted viral epitope. *J. Exp. Med. 186:1481.*
19. Dadaglio, G., C. A. Nelson, M. B. Deck, S. J. Petzold, and E. R. Unanue. 1997. Characterization and quantitation of peptide-MHC complexes produced from hen egg lysozyme using a monoclonal antibody. *Immunity 6:727.*
20. Porgador, A., J. W. Yewdell, Y. Deng, J. R. Bennink, and R. N. Germain. 1997. Localization, quantitation, and in situ detection of specific peptide-MHC class I complexes using a monoclonal antibody. *Immunity 6:715.*
21. Bender, A., L. K. Bui, M. A. Feldman, M. Larsson, and N. Bhardwaj. 1995. Inactivated influenza virus, when presented on dendritic cells, elicits human $CD8^+$ cytolytic T cell responses. *J. Exp. Med. 182:1663.*
22. Flynn, K. J., G. T. Belz, J. D. Altman, R. Ahmed, D. L. Woodland, and P. C. Doherty. 1998. Virus-specific $CD8^+$ T cells in primary and secondary influenza pneumonia. *Immunity 8:683.*
23. Lanzavecchia, A. 1990. Receptor-mediated antigen uptake and its effect on antigen presentation to class II-restricted T lymphocytes. *Annu. Rev. Immunol. 8:773.*