COOPERATION BETWEEN CYTOTOXIC AND HELPER T LYMPHOCYTES IN PROTECTION AGAINST LETHAL SENDAI VIRUS INFECTION

Protection by T cells is MHC-restricted and MHC-regulated; a model for MHC–Disease associations

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The hallmark of T lymphocyte recognition is combined specificity for self MHC molecules and nominal antigen, i.e., MHC-restricted recognition. As a rule, cytotoxic T lymphocytes (Tc)[1] are restricted by class I MHC molecules (K, D, and L molecules in the mouse) (1, 2), whereas T helper lymphocyte (Th) recognition is restricted by class II MHC molecules (I-A and I-E molecules in the mouse) (3, 4). Both class I and class II molecules serve as immune response gene products regulating the magnitude of T cell responses (3–13).

Immune regulation by MHC molecules might be an important mechanism to explain MHC-disease associations, although solid evidence for this, especially in HLA–disease associations, is meager.

The opportunity to study the in vivo importance of class I MHC regulation of the Tc response to a natural pathogenic agent of high virulence came with our previous demonstration (14) of a major difference in the capacity to generate a Sendai virus–specific Tc response between C57BL/6 (B6; H-2^b) mice and H-$2K^b$ mutant B6.C-H-2^{bm1} (bm1) mice. These mice differ from each other only in three amino acids in the H-$2K^b$ molecule (15, 16). B6 mice are responders to Sendai virus measured by the generation of virus-specific Tc, virus-specific Th proliferation, and NK cell activity in vitro, and by antiviral antibody production and virus-specific delayed type hypersensitivity (DTH) in vivo (14, 17, and this study). In contrast, bm1 mice are Tc nonresponders against this virus, but do not differ in any of the other immune parameters (14, 17, and this study). These immune parameters were also studied in thymus deficient *nu/nu* mice. These mice show not only deficient Tc responses, but also deficient Th responses and

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[1] *Abbreviations used in this paper:* DTH, delayed-type hypersensitivity; HAU, hemagglutinating unit; i.n., intranasal; LCMV, lymphocytic choriomeningitis virus; Tc, cytotoxic T lymphocyte.

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thus deficient DTH and antibody responses, although their baseline NK activity is increased in comparison with normal mice (14, 17 and this study).

We now report that B6, bm1, and B6 *nu/nu* mice differ from each other in susceptibility to lethal pneumonia induced by intranasal (i.n.) inoculation of virulent Sendai virus. T cell–deficient B6 *nu/nu* animals were most susceptible to the lethal effects of the virus, whereas B6 mice were most resistant. bm1 mice showed intermediate susceptibility. The difference in susceptibility between B6 and bm1 mice is ascribed to the defective Sendai-specific Tc response in bm1 mice. The importance of Tc was further demonstrated by the complete protection of B6 *nu/nu* mice (in an H-2K^b-restricted manner) against a lethal virus dose by i.v. injection of an IL-2-dependent, Sendai-specific, and H-2K^b-restricted B6 Tc clone. Thus, in this case, an association between MHC and susceptibility to a natural pathogenic agent of high virulence is linked to Tc nonresponsiveness on the basis of class I MHC regulation. Apart from Tc, an important role for virus-specific Th cells in the protection against the virus is evident from the difference in susceptibility between bm1 and B6 *nu/nu* mice. This conclusion was supported by the demonstration that inoculation of I-A^b-restricted Sendai-specific Th clones clearly prolonged the lifespan of B6 *nu/nu* mice and bm1 *nu/nu* mice in an I-A-restricted manner, after challenge with a lethal dose of virus, and by the demonstration that inoculation of these Th clones provided help to virus-specific Tc. This study provides a unique model for the further investigation of MHC–disease associations, and shows that antigen-specific Tc and Th cells cooperate in in vivo protection against virus infection.

Materials and Methods

Animals. Normal C57BL/6, 129/J, B10.A(4R), B10.A(5R), and B10.MBR mice, and H-2 mutant B6.C-H-2^{bm1} (bm1) and B6.C-H-2^{bm12} (bm12) mice and C57BL/6 *nu/nu* mice were bred in our laboratory under specific pathogen-free conditions. bm1 *nu/nu* mice were bred as described previously (17). bm12 *nu/nu* mice were bred by mating normal bm12 with C57BL/6 *nu/nu* mice. The F_1 mice were mated and the F_2 *nu/nu* mice were H-2 typed by cytofluorographic analysis on LPS-induced (30 μg/ml), 4-d-cultured lymphoblasts obtained from lymph node cells. For typing, we used mAb 34-5-3S, a gift from Dr. D. H. Sachs (National Institutes of Health, Bethesda, MD), which reacts strongly with the lymphocytes of I-A^b but not of I-A^{bm12} mice (18). The mouse strains used in this study, their H-2 haplotypes, and the structural alterations in the H-2 molecules of the mutant mouse strains are listed in Table I. All mice were used at 6–12 wk of age.

Viruses. Nonvirulent Sendai virus, lot 40340087, was obtained from Flow Laboratories (McLean, VA). This virus had been propagated in pathogen-free eggs. The titer was 10^4 hemagglutinating units (HAU) per milliliter. Virulent Sendai virus, strain P3193, was a gift of Dr. J. C. Parker (Microbiological Associates, Bethesda, MD). This virus was propagated in the lungs of 129/J mice. Homogenates of infected lungs (10% suspension, wt/vol, in basal [Eagle's] medium with antibiotics, clarified at 2,300 *g* for 20 min at 4°C) were inoculated by i.n. instillation of 0.02 ml of 10% suspension of infected mouse lung cells. Lungs of mice were harvested 6 d after inoculation. P3193 virus was tested by the mouse antibody-production test (20), and found not to contain other murine virus contaminants. The titer was 3.2×10^6 50% tissue culture infective dose ($TCID_{50}$) per milliliter. Both viruses were stored at −70°C.

Virus Titration and Quantitation. Virus titration and quantitation were performed essentially as described (21) with slight modification. For virus titration in tissue culture, clarified 10% suspensions of infected lung cells were diluted 10-fold, and 0.1 ml was inoculated into each of four 2-ml wells of a 24-wells plate (Nunc, Roskilde, Denmark)

TABLE I
H-2 Haplotypes of Mouse Strains Used in This Study

Mouse strain	Abbreviation	H-2 K	H-2 I-A	H-2 D	Structural alteration
C57BL/6	B6	b	b	b	—
C57BL/6 *nu/nu*	B6 *nu/nu*	b	b	b	—
129/J	129/J	b	b	b	—
B10.A(4R)	4R	k	k	b	—
B10.A(5R)	5R	b	b	d	—
B10.MBR	MBR	b	k	q	—
B6.C-H-2^{bm1}*	bm1	bm1	b	b	Glu → Ala at position 152‡ Arg → Tyr at position 155§ Leu → Tyr at position 156§
B6.C-H-2^{bm1} *nu/nu*	bm1 *nu/nu*	bm1	b	b	Same as bm1
B6.C-H-2^{bm12}*	bm12	b	bm12	b	Ile → Phe at position 67‖ Arg → Glu at position 70‖ Thr → Lys at position 71‖
B6.C-H-2^{bm12} *nu/nu*	bm12 *nu/nu*	b	bm12	b	Same as bm12

* Congenic or coisogenic with C57BL/6 (B6, H-2^b)
‡ According to Pease et al. (15)
§ According to Nairn et al. (15)
‖ According to McIntyre et al. (19)

containing a monolayer of primary rhesus monkey kidney cells (a gift from Dr. M. Jonker, TNO, Rijswijk, The Netherlands). Cells were observed for cytopathic effects daily for 14 d, and end-point titers were calculated by the methods of Kärber (22). Virus titration and quantitation in vivo was done using four mice from each of the strains B6, bm1, or B6 *nu/nu* at each titration point. They were inoculated i.n., each with 0.02 ml of a 3.33-fold virus dilution. The same person (W. M. Kast) made all inoculations throughout the titration experiments. Infected mice were observed for disease and death up to day 28 or longer. During these experiments, mice were housed in an isolator in which the airflow was directed from cages containing mice infected with low doses of virus to cages with mice infected with high doses of virus to avoid contamination. Virus titers are expressed as the number of $TCID_{50}$ per 0.02 ml required to cause a lethal infection (LD_{50}).

Immunizations and Elicitation of DTH. Sendai virus–specific DTH responses were elicited in the ear. Mice were primed with 10^2 HAU noninfectious Sendai virus 6 d before challenge by i.n. instillation of 20 μl of Sendai virus suspension diluted in PBS. The challenge was given by injecting into the dorsal surface of the ear a total volume of 25 μl of either PBS (right ear) or 10^2 HAU Sendai virus diluted in PBS (left ear) using a 50 μl Hamilton syringe fitted with a 30-gauge needle. The same procedure was performed in nonimmunized (naive) control mice. Ear swelling was measured with a Mitutoyo model 102-217 micrometer (Steenkist-Rooymans, Eindhoven, The Netherlands). The percentage increase in ear swelling was calculated as [(thickness of left ear) − (thickness of right ear)]/(thickness of right ear). In another type of experiment, naive mice from various strains were injected in both ears with 2×10^5 cells of a particular T cell clone. Either PBS (right ear) or 10^2 HAU Sendai virus in PBS (left ear) was included in the injection mixture in a total volume of 25 μl.

T Cell Clones. Culture conditions for generation of T cell clones were essentially the same as described before (14). In brief, C57BL/6 and bm1 mice were injected i.p. with 10^2 HAU Sendai virus. 3 wk later, spleen cells of these mice were restimulated in vitro with syngeneic, Sendai virus–coated, irradiated (2,500 rad) spleen cells (stimulator cells) for 5 d. Culture medium consisted of Iscove's modified Dulbecco's medium (IMDM) (Gibco, Grand Island, NY), supplemented with 10% pooled human serum, penicillin (100 IU/ml), streptomycin (100 μg/ml), and 2-ME (2×10^{-5} M). Effector cells were run on a

density gradient (lympholyte M; Cedarlane Laboratories, Hornby, Canada), and cultured again in the same way. This procedure was repeated three times, after which a precursor frequency was established in a limiting-dilution assay, using 96 U-shaped well plates (Titertek; Flow Laboratories, McLean, VA) with 1.5×10^5 Sendai-coated syngeneic stimulator cells and 12.5% rat cell supernatant (RF) as lymphokine source (17) in a total volume of 200 μl culture medium. Wells that showed cell growth at a dilution below 10% of the precursor frequency were transferred into 24-mm Costar wells (Costar, Cambridge, MA). Cells were routinely subcultured once a week. Th clone 6C3, derived from a B6 mouse, and Th clone 1H11, derived from a bm1 mouse, both have the marker profile Thy-1^+, L3T4^+, Lyt 2^-, and produce IL-2 and IFN-γ after Con A stimulation (not shown). Tc clone 5, derived from a B6 mouse, has the marker profile Thy-1^+, L3T4^-, Lyt 2^+. This clone produced IFN-γ, but did not produce IL-2 after Con A stimulation (not shown). They were tested in in vivo and in vitro assays ~2 mo after establishment. Th clone EA-7, specific for egg albumin and derived from a B6 mouse, was originally described by Dr. M. H. Schreier (23), and was obtained through the kind courtesy of Dr. R. Benner and I. S. Klasen (Erasmus University, Rotterdam, The Netherlands).

To explore the protective capabilities of these clones, naive mice were inoculated i.n. with 10 LD_{50} Sendai virus, and 1 h later were given one i.v. injection of cloned T cells in PBS or PBS alone. The results of these tests are given in mean survival time ±SD. For statistical analysis of these results, the Mann-Whitney U test was used.

Recombinant IL-2. Recombinant IL-2 (batch RNB 85738/09Y) was obtained from Biogen (Geneva, Switzerland). In protection experiments, it was administered as a single subcutaneous injection of 1.25×10^4 U/mouse in CFA, with 0.5% BSA.

Proliferative Response of Sendai-specific Th Clones. Th cell clones (10^4) were cultured with 5×10^5 Sendai-modified or nonmodified spleen cells that were irradiated with 2,500 rad in flat-bottomed tissue culture plates (Costar) in a total volume of 0.2 ml culture medium containing 12.5% RF. Cultures were incubated at 37°C and 5% CO_2 for 4 d, the last 4 h in the presence of 0.4 μCi [^{3}H]thymidine added in 20 μl. The cells were harvested with an automated cell harvester, and radioactivity was measured in a liquid scintillation counter. The data presented constitute the mean value of five replicate wells (in cpm).

NK Cell Assay. Varying numbers of spleen cells of different mouse strains were used as effector cells at different times after i.p. inoculation with 3×10^2 HAU Sendai virus against 5×10^3 Na^{51}Cr-labelled YAC-1 target cells in 0.2 ml culture medium in wells of roundbottomed microtiter plates. After 6 h incubation at 37°C in humidified air with 5% CO_2 the percent specific ^{51}Cr release was determined as described (14). Background release was always <15%. The standard error of triplicate cultures was always <3% specific ^{51}Cr release.

Measurement of Tc Effector Activity. Tc effector activity of Tc clone 5 (Tc-5) was essentially measured as described (14). As targets, we used ^{51}Cr-labelled Con A–induced (2.5 μg/ml) lymphoblasts (3-d cultures). Percent specific ^{51}Cr release was determined as described (14). Background release was always <25%. The standard error of triplicate cultures was always <3% specific ^{51}Cr release.

Results

Sendai Virus–specific DTH Response

Primed B6, bm1, and B6 *nu/nu* as well as unprimed (naive) B6 mice were tested for their ability to generate a Sendai virus–specific DTH response. As shown in Fig. 1, unprimed B6 mice could not generate a Sendai virus–specific DTH response. Primed B6 and bm1 mice showed equal percentages of ear swelling after challenge with Sendai virus. Thus, these two mouse strains did not differ in the DTH response to Sendai virus. In contrast, B6 *nu/nu* mice could not generate a Sendai-specific DTH response, illustrating the T cell dependency of the DTH response.

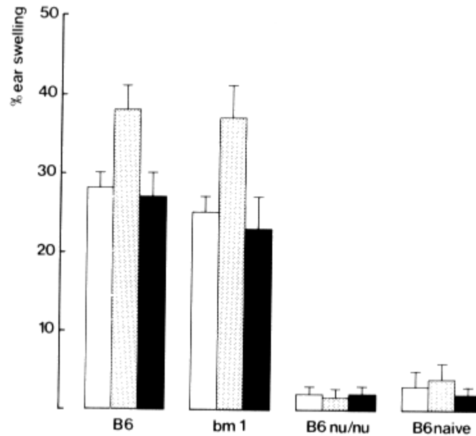

FIGURE 1. Sendai virus-specific DTH response. Mice were naive (not primed) or were primed with 10^2 HAU Flow Sendai virus 6 d before challenge by i.n. instillation of 20 μl of virus suspension diluted in PBS. The challenge was given by injection of a total volume of 25 μl of either PBS (right ear) or 10^2 HAU Flow Sendai virus diluted in PBS (left ear). The same procedure was performed in nonimmunized (naive) control mice. Ear swelling was measured at 24 (white bar), 48 (shaded bar), or 72 h (black bar). Data are expressed as the mean percentage ± SE of 100 × [(left ear thickness) − (right ear thickness)]/(right ear thickness) for groups of 35 B6, 12 bml, 17 B6 *nu/nu*, and 6 B6 naive mice.

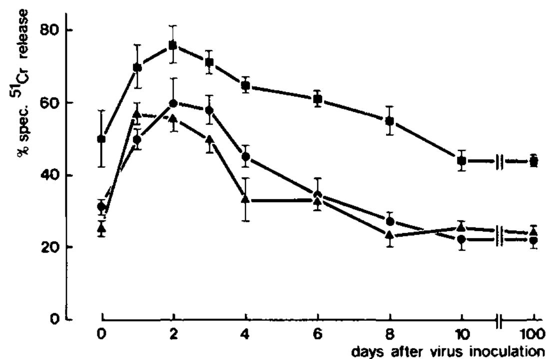

FIGURE 2. Time course of NK activity in spleen cells of different mouse strains after i.p. inoculation with 3 × 10^2 HAU Sendai virus per mouse. Symbols represent NK activity of: ▲, bml mice; ●, B6 mice; ■, B6 *nu/nu* mice. Cytotoxic activity was measured by ^{51}Cr release assay using ^{51}Cr-labelled YAC-1 cells as targets. E/T ratio was 100:1. Each data point represents the mean ± SEM of six mice.

NK Cell Activity

The time course of NK cell activity was studied in naive B6, bml, and B6 *nu/nu* mice after 3 × 10^2 HAU Sendai virus inoculation per mouse (Fig. 2). NK cell activity in control mice was 31 ± 2% specific ^{51}Cr release at an E/T ratio of 100:1 for B6 mice; 26 ± 2% for bml mice, and 50 ± 8% for B6 *nu/nu* mice (mean ±SEM of six mice). The initial elevation in NK cell cytotoxic activity was observed 1 d after infection. Peak levels of NK activity occurred on the second day, and by the eighth day, it had declined to normal levels. B6 and bml mice showed equal kinetics of NK cell activity before and after priming with Sendai virus. In contrast, B6 *nu/nu* mice, though displaying the same time course of

TABLE II
Susceptibility of Different Mouse Strains to Virulent Sendai Virus Infection

Mouse strain*	Number of replicate titrations‡	$LD_{50} \pm SD$§
C57BL/6	4	152.0 ± 74.9
B6.C-H-2^{bm1}	3	14.7 ± 4.8
C57BL/6 *nu/nu*	2	0.5 ± 0.6

* Different groups of naive mice were titrated by i.n. inoculation of 0.02 ml of 3.33-fold virus dilutions.
‡ One titration consisted of at least seven titration points. Four mice were used at each titration point.
§ Values of the mean LD_{50} ± SD expressed in tissue culture infective $dose_{50}$ per 0.02 ml.

NK cell activity, showed a higher basic level of NK activity, with a return to similar values after infection. Thus B6 and bm1 mice did not differ in NK cell activity and NK cell activity kinetics after challenge with Sendai virus, whereas B6 *nu/nu* mice had high baseline NK cell activity before and after challenge with Sendai virus.

Susceptibility to Virulent Sendai Virus Infection

C57BL/6 (B6), H-$2K^b$ mutant bm1, and B6 nude (*nu/nu*) mice were challenged i.n. with virulent Sendai virus to study their susceptibility (Table II). We found that the LD_{50} for the Sendai Tc–deficient bm1 strain was ~10 times lower than for the B6 strain, compatible with an important role of virus-specific Tc. The LD_{50} for the T cell–deficient B6 *nu/nu* strain, in turn, was 30 times lower than for the bm1 strain, indicating an important role of T cells other than Tc, notably Th cells, in antiviral immunity. To further confirm the notion that both Th and Tc cells are important in antiviral immunity, nude mouse protection experiments with Th and Tc clones were carried out.

Characterization of Sendai Virus–specific Th and Tc Clones

Sendai virus–specific Th clones are class II (I-A^b)-restricted. The capacity of Th-6C3 and -1H11 to proliferate in a Sendai virus–specific manner was tested against Sendai-coated stimulator cells of various H-2 types. Table III illustrates that both clones specifically proliferate to Sendai virus, but only against I-A-type stimulator cells. Non-I-A^b B10 MBR-, and bm12 (I-A^b mutant) Sendai-coated stimulator cells were nevertheless able to stimulate an H-$2K^b$-restricted Sendai-specific Tc line (not shown), thereby excluding the possibility that these cells failed to express Sendai virus antigens. Thus, Th-6C3 and -1H11 are I-A^b-restricted.

Sendai virus–specific DTH responses mediated by Th clones are also class II (I-A^b)-restricted. The ability of Th-6C3 to function in vivo was shown by the induction of a Sendai-specific DTH response. Fig. 3 shows that Th-6C3 is capable of inducing a DTH response to Sendai virus only when the host has MHC determinants of the I-A^b type. The possibility that the presence of allodeterminants on either the T cell clone or the host suppressed a potential response in I-A^b-disparate MBR, 4R mice and bm12 was ruled out, because 5R mice also have

TABLE III

Sendai Virus-specific and I-A^b-restricted Proliferative Response of T_h Clones

Clone*	[^{3}H]Thymidine uptake (mean cpm) with various stimulators‡												
	B6S (bbb)	B6	bm1S (bm1bb)	bm1	bm12S (bbm12b)	bm12	B10.MBRS (bkq)	B10.MBR	4RS (kkb)	4R	5RS (bbd)	5R	RF§
T_h-6C3	13,677	1,415	14,128	2,013	1,093	1,422	315	490	373	367	10,371	1,196	584
T_h-1H11	8,734	342	9,951	305	211	245	30	46	390	199	8,860	327	67

* 10^4 T_h clone cells were cultured for 4 d in the presence of stimulator cells.

‡ Stimulator cells (5×10^5) were 2,500 rad irradiated Sendai-coated (S) or uncoated spleen cells. All stimulations were carried out in the presence of 12.5% RF as lymphokine source. Data are expressed as mean cpm ^{3}H uptake tested in triplicate.

§ The proliferative response in the presence of 12.5% RF only was used as background stimulation.

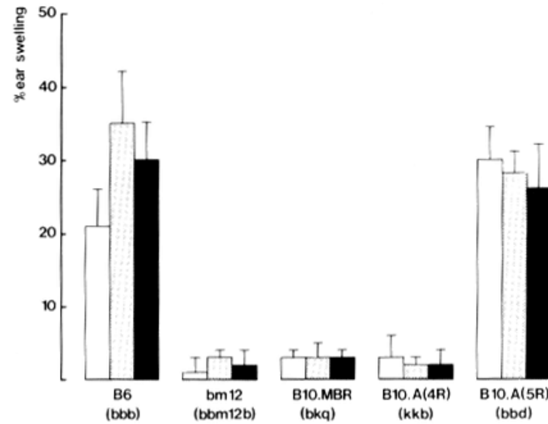

FIGURE 3. DTH response elicited by Th clone 6C3. Th clone cells (2×10^5) were injected into both ears of naive mice of the strains indicated. Naive mice by themselves cannot generate a DTH response to Sendai virus (Fig. 1). Either PBS (right ear) or 10^2 HAU flow Sendai virus in PBS (left ear) was included in the injection mixture in a total volume of 25 μl. Ear swelling was measured at 24 (white bar), 48 (shaded bar), or 72 h (black bar). Data are expressed as the mean percentage ± SE of 100 × [(left ear thickness) − (right ear thickness)]/(right ear thickness) for groups of five mice.

TABLE IV
Sendai Virus–specific and K^b-restricted Killing Activity of Tc Clone 5

Clone	Percent specific ^{51}Cr release using target cells at E/T ratios of 5:1*							
	B6S (bbb)[‡]	B6	bm1S (bm1bb)	bm1	4RS (kkb)[§]	4R	5RS (bbd)	5R
Tc-5	56	0	4	7	1	2	51	0

* Mean of triplicate determinations. 10^3 Con A blasts used.
[‡] S, Sendai virus–infected.
[§] 4R Sendai virus–infected cells are always killed by syngeneic anti-Sendai effector cells (not shown).

multiple H-2 differences from the B6-derived T cell clone. These data show that the in vivo restriction specificity of this Th clone is also I-A^b encoded. Similar results were obtained with the bm1 Th-1H11 (not shown).

Sendai virus–specific Tc clone is class I (K^b)-restricted. The capacity of Tc-5 to generate Sendai-specific lytic activity was demonstrated against Sendai-infected target cells of various H-2 types. Table IV shows that Tc-5 specifically killed Sendai-infected targets only when they expressed the K^b molecule. This Tc clone was further characterized by the ability to induce a Sendai-specific DTH response, and by its ability to proliferate in vitro in a Sendai-specific manner only against K^b-type stimulator cells (not shown). These data show that the restriction specificity of Tc-5 is K^b.

Protection Against Lethal Sendai Virus Infection in nu/nu Mice by T Cell Clones

In three separate experiments (shown in Tables V, VI, and VII), we determined whether these clones could protect against a lethal Sendai virus infection. Therefore, B6 *nu/nu*, bm1 *nu/nu*, and bm12 *nu/nu* mice were given a Sendai virus dose of 10 LD_{50} i.n., and simultaneously, T cell clones or PBS i.v. Tables

TABLE V

Protection against Lethal Sendai Infection in Different Mouse Strains by Th Clones (Exp. 1)

Mouse strain	Number of mice tested	Cells injected (number of cells)*	Survival time (mean ± SD)	Significance (*p*)‡	Range
			d		*d*
B6 *nu/nu*	24	PBS	19.6 ± 5.4	<0.001	11–28
	3	Th-6C3 (10^7)	57.3 ± 6.4		50–62
bm12 *nu/nu*	6	PBS	21.3 ± 5.5	>0.1	14–27
	3	Th-6C3 (10^7)	18.6 ± 3.2		15–21
bm1 *nu/nu*	6	PBS	15.5 ±3.0	0.012	11–20
	3	Th-1H11 (10^7)	48.3 ± 9.0		39–57
B6 +/+	20	PBS	8.0 ± 1.4	>0.1	6–12
	3	Th-6C3 (10^7)	9.0 ± 1.7		8–11
bm1 +/+	20	PBS	8.1 ± 2.1	>0.1	6–14
	3	Th-1H11 (10^7)	8.0 ± 1.0		7–9

* Different groups of naive mice were inoculated i.n. with 10 LD_{50} Sendai virus and 1 h later were given one injection i.v. with T cell clones, diluted in PBS, or only PBS. Th-6C3 is a Sendai virus–specific B6 Th clone. Th-1H11 is a Sendai virus–specific bm1 Th clone.

‡ Mann-Whitney U test vs. mice injected with PBS.

TABLE VI

Protection against Lethal Sendai Virus Infection in Different Mouse Strains by Th and Tc Clones (Exp. 2)

Mouse strain	Number of mice tested	Cells injected (number of cells)*	Survival time (mean ± SD)	Significance (*p*)‡	Range
			d		*d*
B6 *nu/nu*	6	PBS	18.8 ± 5.0		13–25
	3	Th-6C3 (10^7)	35.7 ± 4.9	0.012	30–39
	3	Th-6C3 (3×10^6)	33.7 ± 3.8	0.012	31–38
	3	Th-6C3 (10^6)	25.3 ± 10.8	>0.1	13–33
	3	Tc-5 (3×10^6)	25.0 ± 1.0	0.024	24–26
	3	Tc-5 (3×10^6) + Th-6C3 (3×10^6)	>100.0	0.012	—
	3	Tc-5 (3×10^6) + rIL-2	>100.0	0.012	—
	4	Th-EA-7 (10^7) + rIL-2	21.2 ± 3.8	>0.1	16–25
bm12 *nu/nu*	4	Th-6C3 (10^7)	20.1 ± 5.3	>0.1	13–25

Where indicated, mice were also injected s.c. with 1.25×10^4 U rIL-2. Tc-5 is a Sendai virus–specific B6 Tc clone, and Th-EA-7 is an egg albumin–specific B6 Th clone.

* As in Table IV.

‡ Mann-Whitney U test vs. B6 *nu/nu* mice injected with PBS.

V, VI, and VII show that B6 *nu/nu* mice die between days 15 and 19. Injection of B6 Th-6C3 extended the survival time of B6 *nu/nu* mice by a factor of two (Tables VI and VII) or three (Table V). This effect was dose dependent (Table VI). Although life prolongation was reached with Sendai-specific Th clones in all three experiments, no permanent survivors were observed. The I-A^b restric-

TABLE VII

Protection against Lethal Sendai Virus Infection in Different Mouse Strains by Th and Tc Clones (Exp. 3)

Mouse strain	Number of mice tested	Cells injected (number of cells)*	Survival time (mean ± SD)	Significance (p)‡	Range
			d		*d*
B6 *nu/nu*	8	PBS	15.5 ± 2.6		13–20
	4	Th-6C3 (3×10^6)	30.3 ± 7.6	0.002	21–37
	4	Tc-5 (3×10^6)	23.5 ± 1.7	0.002	22–25
	4	Th-6C3 (3×10^6) + Tc-5 (3×10^6)	>50.0§	0.002	—
	4	Th-6C3 (3×10^6) + Tc-5 (10^6)	31.5 ± 9.0	0.002	21–39
	4	rIL-2	16.7 ± 2.4	>0.1	15–20
bm1 *nu/nu*	4	Tc-5 (3×10^6) + rIL-2	13.7 ± 2.9	>0.1	12–18

* As in Table V.

‡ Mann-Whitney U test vs. B6 *nu/nu* mice injected with PBS.

§ One mouse died on day 26, the other three remained healthy beyond day 50.

TABLE VIII

Summary of Immune Responses against Sendai Virus in Different Mouse Strains

Assay of immune response	Mouse strain			Reference
	B6	bm1	B6 *nu/nu*	
In vitro				
Tc	+	–	–	14, 17
T cell proliferation	+	+	–	14, 17
NK	+	+	++	This study
In vivo				
Antibody production	+	+	–	14, 17
DTH	+	+	–	This study
Resistance against infection	+	±	–	This study

tion specificity of Th-6C3 was again apparent in vivo, because bm12 *nu/nu* mice could not be protected from death by this clone (Tables V and VI). Analogous injection of bm1 Th-1H11 into bm1 *nu/nu* mice could also significantly extend their survival time (Table V). Injection, however, of an I-A^b-restricted B6 Th clone specific for egg albumin had no protective effect (Table VI), illustrating that the life prolongation by the Sendai-specific Th clones was specific. The mean survival time of normal B6 and bm1 mice could not be extended by i.v. injection with Th-6C3 or -1H11, respectively (Table V), probably because the LD_{50} virus dose for T cell–competent animals is so much higher that much higher doses of cloned T cells would be required for measurable protection. The Sendai virus–specific B6 Tc-5 by itself only marginally but significantly extended the survival time of B6 *nu/nu* mice (Tables VI and VII). However, upon injection of a combination of 3×10^6 Tc-5 and 3×10^6 Th-6C3 cloned T cells, almost all mice survived and remained healthy (Tables VI and VII), indicating cooperation between Th and Tc cells in antiviral immunity. Probably Th cells mediate their activity in this cell-cell cooperation at least partially by IL-2 release, because

permanent survival was also achieved by injection of a mixture of 3×10^6 Tc-5 and IL-2 (Table VI). rIL-2, by itself (Table VII) or in conjunction with an irrelevant Th clone (Table VI), had no protective effect. The protective effect of Tc-5 under conditions of sufficient Th supply was dose dependent (Table VII), and the in vitro H-$2K^b$ restriction specificity of Tc-5 was also apparent in vivo because bm1 *nu/nu* mice could not be protected from death by this clone (Table VII) under conditions where B6 *nu/nu* mice could be protected (Table VI).

Discussion

This study establishes a clear association between MHC type and susceptibility to Sendai virus–induced lethal pneumonia. Mutation of three amino acids in the H-$2K^b$ class I molecule in bm1 mice causes a higher susceptibility to the lethal effect of the virus, compared with B6, the strain of origin. The only measurable difference in Sendai-specific immune responses between bm1 and B6 mice is the inability of the mutant to generate Sendai-specific Tc (Table VIII). This difference is also the only expected difference, because class I MHC molecules only restrict and regulate Tc responses, and because no other genetic differences between B6 and bm1 mice are known to exist. Indeed, all other immune responses, such as DTH (Fig. 1), T cell proliferation (Table VIII), and antibody production (Table VIII), as well as NK activity (Fig. 2) of these two mouse strains were identical. The likelihood that the increased virus susceptibility and the Tc nonresponsiveness of the bm1 mouse are causally related is further enhanced by the fact that the H-$2K^b$ molecule is the only functional class I MHC restricting element for Sendai virus–specific Tc in B6 mice (14).

The importance of virus-specific Tc in antiviral immunity was further revealed by the fact that B6 *nu/nu* mice could be protected from death by transfer of a B6 Sendai virus–specific Tc clone. A positive control for the importance of Tc against Sendai virus in bm1 mice would be a transfer of bm1 Sendai-specific Tc clones into lethally infected bm1 mice. However, bm1 mice are Tc nonresponders against Sendai virus, therefore, such bm1 Sendai-specific Tc clones are nonexistent, even after prolonged cell culture in vitro with IL-2 (W. M. Kast, unpublished observations). Moreover, transfer of B6 Tc clones specific for Sendai virus into lethally infected bm1 mice is unlikely to have any protective effect because of the MHC restriction of these Tc clones in vitro (Table IV) and in vivo (Table VII).

One of the most striking features of antiviral T cell immunity revealed here is the cooperation between Th and Tc cells in antiviral immunity. Permanent survival of lethally infected *nu/nu* mice was achieved only with a mixture of virus-specific Th and Tc (Tables VI and VII) or a mixture of Tc and rIL-2 (Table VI). The cooperative effect of Th cells can at least partially be explained by the release of IL-2, because (*a*) Th-6C3 and -1H11 produce IL-2, whereas the Tc-5 does not (data not shown), and (*b*) the Th cell in this cell-cell cooperation can be replaced by rIL-2 (Table VI). This type of T-T cooperation is reminiscent of Th/Tc cooperation in the T cell response to MHC alloantigens (24–26) and the male antigen, H-Y (27). IL-2 release does not explain the fact, however, that Th clones by themselves, without Tc, also display a marked protective effect

(Tables V–VII), because rIL-2 by itself (Table VII) or in conjunction with an irrelevant Th clone (Table VI) has no protective effect.

The importance of Th cells was further revealed by the difference in susceptibility (to Sendai virus infection) between T cell–deficient B6 *nu/nu* mice and Tc-deficient bm1 mice (Tables II and VIII). It seems likely that this difference is due (directly or indirectly) to the generation of Sendai-specific Th cells in bm1 mice.

Transfer of immune T cells was shown (28–30) to protect against several viruses such as influenza virus, herpes simplex virus type 1 (31), and lymphocytic choriomeningitis virus (LCMV) (32, 33). Usually, Tc clones were transferred in the same, or even higher amounts than in this study. When Th cells were used, they sometimes worsen (34) or ameliorate (35) virus-induced disease. In our system, the protection by Th and Tc clones against lethal infection could be titrated (Tables VI and VII). Also, as expected from the results of DTH experiments indicating I-A^b restriction in vivo, the Th clones could not protect I-A^b-disparate bm12 *nu/nu* mice from death (Tables V and VI). Furthermore the Tc clone could not protect H-$2K^b$-disparate bm1 *nu/nu* mice from death (Table VII). In vivo protection with immune T cells against virus infection only functions in syngeneic situations (31, 32). In these studies, completely different allogeneic strains were used, so that the exact genetic restriction element was not identified. We now show that the in vivo protection by cloned Th cells shows the same I-A^b restriction as in vitro proliferation and in vivo DTH reaction, and that the in vivo protection by cloned Tc cells shows the same H-$2K^b$ restriction as in vitro cell-mediated cytotoxicity.

The exact mechanism(s) by which both Th and Tc clones exert their protection remain(s) to be solved, but (Table V [Exp. 1]) no Sendai-specific Tc or Sendai-specific antibody production was demonstrable in *nu/nu* mice 42 d after injection of the Th clones alone, whereas at 40 d after Th clone transfer, these mice were still capable of generating Sendai-specific DTH reactions (data not shown). One possible protective mechanism of Th and Tc is production of IFN-γ, which is released by both our Th and Tc clones (data not shown). In experiments with influenza virus (29) and with herpes virus (31), only those Tc clones that produced IFN-γ could clear the virus in vivo. However, recent experiments (30), also in the influenza system, showed that the protective effect of cloned Tc was highly specific and probably not correlated with IFN-γ production. Therefore the straightforward possibility that Tc protect by killing virus-infected cells remains a distinct one, and is currently our favorite.

A direct link between MHC type and susceptibility to acute viral illness has not been established before. On the other hand, the association between MHC type and susceptibility to chronic disease induction by RNA tumor viruses, where immune mechanisms are probably responsible for protection, is well established (36–42). In this case, again, the ability to generate a strong MHC-regulated immune response is associated with resistance to the induction of disease. In lethal LCMV-induced choriomeningitis, however, the generation of a strong MHC-regulated immune response (notably class I–restricted Tc) is associated with susceptibility to the induction of disease. In this model (e.g., intracerebral inoculation of LCM virus), the autoaggressive potential of the antiviral immune

response outweighs the beneficial effects of the T cell response (43–46). Indeed, recently (47), Zinkernagel et al. showed that mice producing Tc against LCMV because they have an appropriate H-2D-restricting element die after infection, whereas mice that do not generate Tc because they lack a proper H-2D-restricting element survive (47). Thus, generation of a strong class I MHC–regulated antiviral Tc response can have two final results: increased resistance to the induction of disease, as demonstrated in this study, or increased susceptibility to the induction of disease (e.g., LCMV) (47). The outcome depends on a series of factors, including cytopathogenicity and virulence of the virus, and the beneficial versus harmful effects of T cell activity (48).

Summary

The in vivo importance of class I MHC regulation of the Tc response to a natural pathogenic agent of high virulence was studied on the basis of our previous demonstration of a major difference in the capacity to generate a Sendai virus–specific Tc response between C57BL/6 (B6, $H\text{-}2^b$) mice and $H\text{-}2K^b$ mutant $B6.C\text{-}H\text{-}2^{bm1}$ (bm1) mice. These two mouse strains differ from each other only in three amino acids in the crucial $H\text{-}2K^b$ restriction element for this response. bm1 mice, in contrast to B6 mice, are Tc nonresponders against this virus, but show Sendai-specific T cell proliferation, antibody production, and DTH reactions, as well as NK cell activity, equal to those of B6 mice.

B6, Sendai Tc-deficient bm1 and T cell–deficient B6 *nu/nu* mice differ from each other in susceptibility to lethal pneumonia induced by i.n. inoculation of virulent Sendai virus. The lethal dose (LD_{50}) in B6 mice averaged 152 $TCID_{50}$, in bm1 mice, 14 $TCID_{50}$ and in B6 *nu/nu* mice 0.5 $TCID_{50}$. The importance of Tc was also shown by the complete protection of B6 *nu/nu* mice against infection with a lethal virus dose by i.v. injection of a Sendai virus–specific, IL-2-dependent and $H\text{-}2K^b$-restricted B6 Tc clone. In vivo protection by this Tc clone was $H\text{-}2K^b$-restricted.

Apart from Tc, an important role for virus-specific Th cells is evident from the difference in susceptibility between bm1 and B6 *nu/nu* mice. This conclusion was supported by the demonstration that the mean survival time of B6 *nu/nu* and bm1 *nu/nu* mice could be significantly prolonged, in an $I\text{-}A^b$-restricted manner, by the injection of in vitro–propagated, Sendai-specific B6 or bm1 Th clones after a lethal dose of Sendai virus, and by the demonstration that inoculation of these Th clones provided help to virus-specific Tc by means of IL-2 production. Strikingly, Th and Tc cooperate in anti–Sendai virus immunity, since permanent survival of lethally infected *nu/nu* mice was only achieved by inoculation of a mixture of Tc and Th clones or a mixture of a Tc clone and rIL-2.

This study provides a unique model for the study of MHC–disease associations.

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