Influence of β_2 -Microglobulin Expression on Gamma Interferon Secretion and Target Cell Lysis by Intraepithelial Lymphocytes during Intestinal *Listeria monocytogenes* Infection

MASASHI EMOTO, OLIVER NEUHAUS, YOSHIKO EMOTO, AND STEFAN H. E. KAUFMANN*

Department of Immunology, University of Ulm, D-89070 Ulm, Germany

Received 23 August 1995/Returned for modification 20 October 1995/Accepted 22 November 1995

Numerous microbial pathogens, including *Listeria monocytogenes*, enter the host through the intestine. Although relatively little is known about the biological functions of intestinal intraepithelial lymphocytes (i-IEL), they are generally considered a first line of defense against intestinal infections. In the mouse, the vast majority of i-IEL express the CD8 coreceptor either as a CD8 α/α homodimer or as a CD8 α/β heterodimer. The CD8 coreceptor of T-cell receptor (TcR) γ/δ i-IEL is exclusively homodimeric, whereas the CD8-expressing TcR α/β i-IEL segregate into equal fractions of CD8 α/α and CD8 α/β cells. We infected β_2 -microglobulin (β_2m) $^{+/-}$ mice (possessing all i-IEL populations) and $\beta_2m^{-/-}$ mutant mice (lacking all CD8 α/β $^{+}$ i-IEL and having few CD8 α/α $^{+}$ TcR α/β i-IEL) with *L. monocytogenes* per os and determined their biological functions after TcR ligation with monoclonal antibodies. Cytolytic activities of TcR α/β and TcR γ/δ i-IEL from $\beta_2m^{+/-}$ mice were not influenced by intestinal listeriosis. Cytolytic activities of TcR α/β i-IEL were impaired in uninfected $\beta_2m^{-/-}$ mice, but this reduction was reestablished as a consequence of intestinal listeriosis. Frequencies of gamma interferon (IFN- γ)-producing TcR α/β i-IEL in uninfected $\beta_2m^{-/-}$ mice were reduced, compared with that in their heterozygous controls. Equally low frequencies of IFN- γ -producing TcR γ/δ i-IEL in $\beta_2m^{+/-}$ and $\beta_2m^{-/-}$ mutants were found. Listeriosis increased frequencies of IFN- γ -producing TcR α/β and TcR γ/δ i-IEL in both mouse strains. Most remarkably, the proportion of IFN- γ -producing TcR γ/δ i-IEL was elevated 10-fold in listeria-infected $\beta_2m^{-/-}$ mice. Our findings show that the β_2m -independent CD8 β $^{-}$ i-IEL expressing either TcR α/β or TcR γ/δ are stimulated by intestinal listeriosis independent of regional β_2m expression. We conclude that the three major CD8 $^{+}$ i-IEL populations are stimulated by intestinal listeriosis and that CD8 β $^{-}$ i-IEL compensate for the total lack of CD8 β $^{+}$ i-IEL in $\beta_2m^{-/-}$ mutant mice. Hence, in contrast to the peripheral immune system, which crucially depends on CD8 α/β $^{+}$ TcR α/β lymphocytes, the mucosal immune system can rely on additional lymphocytes expressing the CD8 α/α homodimer.

The intestinal mucosal surface is exposed to a wide variety of microorganisms. The so-called intestinal intraepithelial lymphocytes (i-IEL), which are located between epithelial cells, are considered a first line of defense which prevents translocation of the intestinal flora and interferes with host invasion by microbial pathogens. The i-IEL differ from the lymphocytes of the central immune system in various aspects. In particular, the i-IEL are characterized by a marked preponderance of CD8-expressing cells which represent more than two-thirds of the total i-IEL population (14, 18, 20, 30). In contrast to the CD8 $^{+}$ T lymphocytes of the central immune system which almost exclusively express the heterodimeric CD8 α/β coreceptor, the CD8 $^{+}$ i-IEL segregate into two populations, expressing either a homodimeric CD8 α/α or a heterodimeric CD8 α/β molecule (14, 18). The CD8 α/β molecule is exclusively expressed by T-cell receptor (TcR) α/β i-IEL, whereas the CD8 α/α molecule is present on both TcR α/β and TcR γ/δ i-IEL. In addition, some CD4 $^{+}$ TcR α/β i-IEL, CD4 $^{+}$ CD8 $^{+}$ TcR α/β i-IEL, and CD4 $^{-}$ CD8 $^{-}$ (double negative) TcR γ/δ i-IEL, as well as very few double-negative TcR α/β i-IEL, exist (13, 18). The development and functional activation of CD8 α/β $^{+}$ T lymphocytes depends on surface expression of the major histocompatibility complex class I (MHC I)- β_2 -micro-

globulin (β_2m) complex (46). Therefore, the CD8 α/β $^{+}$ i-IEL are virtually absent from $\beta_2m^{-/-}$ gene disruption mutant mice lacking surface-expressed MHC I- β_2m (49). In contrast, the CD8 α/α -expressing i-IEL develop virtually independently of β_2m , although some influence of β_2m appears likely (7, 31, 35). Thus far, it is unclear whether and how these β_2m -independent T lymphocytes recognize antigenic ligands.

Experimental infection of mice with the intracellular bacterium *Listeria monocytogenes* has been widely used as a model for analyzing the mechanisms underlying cell-mediated immunity against intracellular pathogens (17). Most studies have restricted themselves to systemic infection. However, natural infection with *L. monocytogenes* is food borne, and therefore, i-IEL should provide a first line of defense against listerial invasion (8, 22, 34). Despite their prominent location at the port of microbial entry into the host, relatively little is known about the role of i-IEL in host resistance. It has been shown that i-IEL from normal mice express high cytolytic activities and produce gamma interferon (IFN- γ) either spontaneously or after TcR ligation by monoclonal antibodies (MAb) (10, 42, 44). Although preactivation of i-IEL by the normal intestinal flora has been suggested, the influence of intestinal bacterial invasion on the biological activities of i-IEL remains to be investigated (2).

In the present study, we have analyzed the influence of per os (p.o.) infection with *L. monocytogenes* on the major i-IEL populations by comparing i-IEL activities from $\beta_2m^{-/-}$ and $\beta_2m^{+/-}$ mice after TcR stimulation with specific MAb. The absence of β_2m expression reduced overall cytolytic activities

* Corresponding author. Mailing address: Department of Immunology, University of Ulm, Albert-Einstein-Allee 11, D-89070 Ulm, Germany. Phone: (0731) 502-3361 or (0731) 502-3360. Fax: (0731) 502-3367. Electronic mail address: Stefan.Kaufmann@Medizin.Uni-Ulm.DE.

of and IFN- γ production by TcR α/β i-IEL, probably as a consequence of the loss of CD8 α/β -expressing cells. Intestinal listeriosis increased cytolytic activities and IFN- γ production by TcR α/β and TcR γ/δ i-IEL in $\beta 2m^{-/-}$ mutants, suggesting that intestinal listeriosis activated these i-IEL independently from $\beta 2m$ -contingent antigen presentation. Our findings provide evidence that CD8 α/β^{+} TcR α/β i-IEL, CD8 α/α^{+} TcR α/β i-IEL, and CD8 α/α^{+} TcR γ/δ i-IEL contribute to regional host defense against intestinal infection with bacterial pathogens.

MATERIALS AND METHODS

Animals. Breeding pairs of $\beta 2m^{-/-}$ mice were a kind gift of R. Jaenisch (49). Homozygous or heterozygous mutants on C57BL/6 background (>sixth back cross) of either sex were used at 10 to 12 weeks of age.

Microorganisms and infection. *L. monocytogenes* EGD organisms were maintained virulent by passage in mice. A sample of liver homogenates was grown in tryptic soy broth at 37°C for 18 h, and aliquots were frozen at -70°C until used. The concentration of bacteria was quantitated by plate counts. Mice were starved for 4 h and then infected p.o. with 10^8 *L. monocytogenes* organisms with a gastric tube.

Cell preparation. The i-IEL were prepared as described previously with slight modifications (16). Briefly, a plastic stick was introduced into the lumen of the small intestine, and the gut was fixed with a thread and inverted. The inverted small intestines were placed in a bottle containing Click's RPMI 1640 complete medium, and the bottle was agitated on an orbital shaker at 37°C for 30 min. Supernatants were collected, and the gut tissue was transferred to a 50-ml tube containing 15 ml of medium and vortexed vigorously. This procedure was repeated twice, and supernatants were collected each time. Purified i-IEL were prepared by passage through a 10-ml syringe loosely packed with glass wool (150 mg; Serva Feinbiochemica, Heidelberg, Germany) and separation over discontinuous Percoll density gradients (1.124 g/ml; Seromed, Berlin, Germany). Gradients were prepared in 10-ml centrifuge tubes by layering from the bottom 70% Percoll (2 ml) and then 40% Percoll (3 ml) containing cells. The tubes were then centrifuged at $600 \times g$ for 25 min. The interface between the 40% and the 70% layer containing lymphocytes of >95% cell viability was collected and washed.

MAB. The following hybridomas were used for MAB preparation: anti-TcR α/β MAB (H57-597) (19); anti-TcR γ/δ MAB (GL3) (11); anti-CD3e MAB (145-2C11) (21); anti-CD8 β MAB (H35-17.2) (9); anti-IFN- γ MAB (R4-6A2 [39] and AN18.17.24 [32]). Hybridoma cells were cultured in medium containing 2% fetal calf serum, and supernatants were collected. The MAB were then concentrated by ammonium sulfate precipitation and purified by affinity chromatography on protein A or G Sepharose (Pharmacia, Freiburg, Germany). Anti-TcR α/β MAB, anti-TcR γ/δ MAB, and anti-IFN- γ MAB (AN18.17.24) were conjugated with biotin. Anti-CD8 β MAB were conjugated with fluorescein isothiocyanate by conventional methods. Phycoerythrin-conjugated anti-CD4 MAB (H129.19) and streptavidin-conjugated-Red 670 were purchased from Gibco (Gaithersburg, Md.). FITC-conjugated anti-CD8 α MAB (YTS 169.4) were obtained from Medac (Hamburg, Germany). Normal hamster immunoglobulin (Ig) was purchased from Dianova (Hamburg, Germany).

Redirected cytolytic assay. The i-IEL were assayed for cytolytic activities against ^{51}Cr -labelled P815 target cells as described previously (44, 47). Briefly, serial dilutions of i-IEL were incubated in 96-well V-bottom plates with 10^3 ^{51}Cr -labelled P815 target cells in the presence or in the absence of anti-CD3 MAB, anti-TcR α/β MAB, anti-TcR γ/δ MAB, or normal hamster Ig at 2 $\mu\text{g}/\text{ml}$ for 4 h. After incubation, 100 μl of supernatants was collected from each well and radioactivity was determined with a gamma counter (Cobra, Canberra Packard, Germany). Percent specific killing was calculated as follows: [(experimental ^{51}Cr release - spontaneous ^{51}Cr release)/(maximum ^{51}Cr release - spontaneous ^{51}Cr release)] $\times 100$. Spontaneous release was always <10% of total ^{51}Cr incorporated into target cells.

ELISPOT assay. MAB-facilitated IFN- γ production by i-IEL was measured by the enzyme-linked immunospot (ELISPOT) assay as described previously (24, 43). Briefly, ELISPOT plates (Millipore, Eschborn, Germany) were coated with anti-IFN- γ MAB (R4-6A2, 1 $\mu\text{g}/\text{ml}$) in 100 μl of 0.05 M carbonate buffer (pH 9.6) per well at 4°C overnight. The coating buffer was then removed, and the plates were dried at room temperature for 1 h. The plates were washed twice with phosphate-buffered saline (PBS) and blocked with PBS containing 1% bovine serum albumin (BSA) at 37°C for 2 h. After the plates were washed twice with PBS, various concentrations of i-IEL and 10^4 P815 cells were cultured at 37°C overnight with 2 μg of anti-CD3 MAB, anti-TcR α/β MAB, anti-TcR γ/δ MAB, normal hamster Ig, or medium alone per ml. The plates were then washed 10 times with PBS and 10 times with PBS containing 0.05% Tween 20 (washing buffer) and incubated with 0.25 μg of biotin-conjugated anti-IFN- γ MAB (AN-18.17.24) per ml at 37°C for 2 h. The plates were then washed 20 times with washing buffer, and streptavidin-conjugated alkaline phosphatase (1:20,000 in PBS containing 0.1% BSA) was added. After 1 h of incubation at 37°C, the plates were washed eight times with washing buffer and twice with alkaline phosphatase buffer. The substrate, 5-bromo-4-chloro-3-indolyl phosphate (Fluka, Basel, Swit-

zerland), and the catalyst, nitroblue tetrazolium chloride (Fluka), were then added, and the plates were incubated at 37°C in the dark for 15 min. The plates were washed with distilled water and dried, and spots were counted by using a dissecting microscope.

Flow cytometry. Single-cell suspensions were stained with fluorescein isothiocyanate-conjugated MAB, phycoerythrin-conjugated MAB, and biotin-conjugated MAB, followed by staining with streptavidin-conjugated Red 670. All incubation steps were performed at 4°C for 30 min, and all washing steps were performed with PBS containing 2.5% normal human serum and 0.1% sodium azide. After being stained, the cells were fixed with 1% paraformaldehyde (Serva) and 10^4 cells were analyzed with a FACScan (Becton Dickinson, Sunnyvale, Calif.) equipped with a data lister and Lysis II software. Live cells were gated by forward and side scatter. Analysis gate was set on TcR α/β or TcR γ/δ cells, and CD4/CD8 expression was displayed.

Histological study. Numbers of i-IEL per 10^3 epithelial cells were counted microscopically in tissue sections of the duodenum after staining with periodic acid-Schiff stain (PAS).

Statistical analysis. Data from different experimental groups were compared by Student's *t* test.

RESULTS

Influence of intestinal listeriosis on IFN- γ -producing i-IEL from $\beta 2m^{-/-}$ and $\beta 2m^{+/-}$ mice. We assessed frequencies of IFN- γ -producing i-IEL from $\beta 2m^{-/-}$ and $\beta 2m^{+/-}$ mice during intestinal *L. monocytogenes* infection. The i-IEL were stimulated with anti-CD3 MAB, anti-TcR α/β MAB, or anti-TcR γ/δ MAB in the presence of P815 cells. High frequencies of IFN- γ -producing i-IEL were induced in uninfected $\beta 2m^{+/-}$ mice by anti-CD3 MAB or anti-TcR α/β MAB, whereas in i-IEL from $\beta 2m^{-/-}$ mice, numbers of IFN- γ producers were significantly ($P < 0.01$) decreased (Table 1). Anti-TcR γ/δ MAB caused a slight though statistically significant ($P < 0.05$) increase in IFN- γ production in both $\beta 2m^{+/-}$ and $\beta 2m^{-/-}$ mice. Thus, the few CD8 β^{-} TcR α/β i-IEL present in $\beta 2m^{-/-}$ mutants were potent IFN- γ producers, whereas only few TcR γ/δ i-IEL produced IFN- γ in response to TcR ligation, regardless of the presence or absence of $\beta 2m$. Consistent with results obtained with C57BL/6 mice (47), frequencies of spontaneous IFN- γ -producing i-IEL were significantly ($P < 0.01$) elevated during intestinal *L. monocytogenes* infection (Table 1). CD3 or TcR α/β ligation by specific MAB increased frequencies of IFN- γ producers in *L. monocytogenes*-infected $\beta 2m^{+/-}$ and $\beta 2m^{-/-}$ mice ($P < 0.01$). These findings suggest that in immunocompetent mice, both CD8 β^{+} and CD8 β^{-} TcR α/β i-IEL are potent IFN- γ producers and that in both i-IEL populations IFN- γ secretion is further stimulated by intestinal listeriosis. Most remarkably, *L. monocytogenes* infection caused a 10-fold increase ($P < 0.001$) in the frequencies of IFN- γ -producing TcR γ/δ i-IEL in $\beta 2m^{-/-}$ mutants, whereas in $\beta 2m^{+/-}$ mice only a slight increase (<3-fold, $P < 0.05$) was observed. As a result, during listeriosis, frequencies of IFN- γ -producing TcR γ/δ i-IEL were higher in $\beta 2m^{-/-}$ mice than in $\beta 2m^{+/-}$ mice. We conclude that intestinal listeriosis activates IFN- γ secretion in all major i-IEL populations. Although we assume that listerial invasion activated IFN- γ -producing TcR γ/δ i-IEL more potently in the absence than in the presence of $\beta 2m$, we cannot exclude a compensatory increase in the frequency of IFN- γ -producing TcR γ/δ i-IEL in $\beta 2m^{-/-}$ mutants.

Influence of intestinal listeriosis on target cell lysis by i-IEL from $\beta 2m^{-/-}$ and $\beta 2m^{+/-}$ mice. Next, the effect of *L. monocytogenes* invasion on cytolytic activities of i-IEL from $\beta 2m^{-/-}$ and $\beta 2m^{+/-}$ mice were studied in MAB-facilitated ^{51}Cr release assays by using MAB with a specificity for CD3, TcR α/β , or TcR γ/δ . Consistent with previous findings (11, 12), strong cytolytic activities were induced in i-IEL from $\beta 2m^{+/-}$ mice, and TcR α/β i-IEL were more potent than TcR γ/δ i-IEL ($P < 0.01$) (Fig. 1). In $\beta 2m^{+/-}$ controls, target cell lysis by TcR α/β and by TcR γ/δ i-IEL remained virtually unchanged under intestinal *L. monocytogenes* infection. Cytolytic activities of TcR α/β i-IEL

TABLE 1. Enumeration of IFN- γ -producing cells among i-IEL from $\beta 2m^{-/-}$ and $\beta 2m^{+/-}$ mice and influence of intestinal *L. monocytogenes* infection^a

Stimulation	IFN- γ -producing cells/ 10^6 i-IEL			
	Uninfected		Infected	
	$\beta 2m^{+/-}$	$\beta 2m^{-/-}$	$\beta 2m^{+/-}$	$\beta 2m^{-/-}$
Anti-CD3 MAb	14,500 $\pm$ 1,852 ^b	6,813 $\pm$ 744 ^{b,c}	32,100 $\pm$ 5,242 ^{b,c}	13,600 $\pm$ 1,633 ^{b,d}
Anti-TcR α/β MAb	13,600 $\pm$ 1,943 ^b	5,363 $\pm$ 791 ^{b,c}	27,000 $\pm$ 4,650 ^{b,c}	12,575 $\pm$ 1,572 ^{b,d}
Anti-TcR γ/δ MAb	525 $\pm$ 82 ^e	613 $\pm$ 44 ^e	1,375 $\pm$ 252 ^{c,e}	5,975 $\pm$ 698 ^{b,f}
Hamster Ig	250 $\pm$ 30	225 $\pm$ 14	850 $\pm$ 103 ^c	925 $\pm$ 171 ^d
P815 alone	263 $\pm$ 18	250 $\pm$ 22	800 $\pm$ 105 ^c	863 $\pm$ 108 ^d
None	225 $\pm$ 28	225 $\pm$ 19	738 $\pm$ 111 ^c	800 $\pm$ 118 ^d

^a Mice were infected p.o. with 10^8 *L. monocytogenes* organisms and i-IEL were prepared on day 0 and day 8 postinfection. The i-IEL (10^6) and P815 cells (10^4) were incubated with MAb overnight in ELISPOT plates coated with anti-IFN- γ MAb. Shown are representative results from three different experiments. Results are means $\pm$ standard deviations of triplicate cultures. Mean numbers of spots per 10^6 cells in controls: without coating anti-IFN- γ MAb, 25 $\pm$ 5; without biotin-conjugated MAb, 38 $\pm$ 6; P815 alone, 63 $\pm$ 10.

^b Significant difference relative to unstimulated i-IEL at $P < 0.001$ at $P < 0.01$.

^c Significant difference relative to uninfected $\beta 2m^{+/-}$ mice.

^d Significant difference relative to uninfected $\beta 2m^{-/-}$ mice at $P < 0.01$.

^e Significant difference relative to unstimulated i-IEL at $P < 0.05$.

^f Significant difference relative to uninfected $\beta 2m^{-/-}$ mice at $P < 0.001$.

were markedly reduced in uninfected $\beta 2m^{-/-}$ mice ($P < 0.01$), whereas those of TcR γ/δ i-IEL remained unaffected by the $\beta 2m$ deficiency. Intestinal *L. monocytogenes* infection, however, strongly increased target cell lysis by TcR α/β and TcR γ/δ i-IEL from $\beta 2m^{-/-}$ mutants ($P < 0.01$) (Fig. 1). Our results suggest that intestinal listeriosis stimulated cytolytic activities in CD8 β^- TcR α/β and TcR γ/δ i-IEL from $\beta 2m^{-/-}$ mice.

Time course of i-IEL activation from *L. monocytogenes*-infected $\beta 2m^{-/-}$ and $\beta 2m^{+/-}$ mice. The $\beta 2m^{-/-}$ and $\beta 2m^{+/-}$ mice were infected p.o. with *L. monocytogenes*, and i-IEL were prepared at different time points thereafter. As shown in Fig. 2, target cell lysis by TcR α/β and TcR γ/δ i-IEL remained virtually unchanged in $\beta 2m^{+/-}$ mice during the course of infection. In contrast, intestinal *L. monocytogenes* infection gradually increased cytolytic activities by TcR α/β and TcR γ/δ i-IEL in $\beta 2m^{-/-}$ mice. Although frequencies of IFN- γ -producing TcR α/β and TcR γ/δ i-IEL were instantaneously elevated by intestinal listeriosis in both $\beta 2m^{-/-}$ and $\beta 2m^{+/-}$ mice, listeriosis had the strongest effects on IFN- γ production by TcR γ/δ i-IEL from $\beta 2m^{-/-}$ mice: already at day 2 of infection, a 10-fold increase was observed. Thus, activation of IFN- γ -producing TcR γ/δ i-IEL preceded stimulation of cytolytic TcR α/β i-IEL in *L. monocytogenes*-infected $\beta 2m^{-/-}$ mutant mice.

Numbers and phenotypes of i-IEL from *L. monocytogenes*-infected $\beta 2m^{-/-}$ and $\beta 2m^{+/-}$ mice. The influence of $\beta 2m$ expression and of intestinal listeriosis on numbers and phenotypes of i-IEL was assessed. The ratio of the total i-IEL to epithelial cells was determined first. Duodenal tissue sections were stained with PAS, in each section 10^3 epithelial cells were counted, and numbers of lymphoid cells within the epithelial layer inside the PAS-positive basal lamina were determined. In $\beta 2m^{+/-}$ mice, 14.2% of total epithelial cells were i-IEL, and intestinal listeriosis slightly increased this proportion to 16.6%. In $\beta 2m^{-/-}$ mutants, the percentage of i-IEL among total epithelial cells was modestly increased from 13.6% to 18.0% during infection. In both cases, however, these increases were not statistically significant. In contrast, total cell numbers recovered after preparation of i-IEL were decreased during intestinal *L. monocytogenes* infection from 11.0×10^6 to 5.8×10^6 i-IEL in $\beta 2m^{+/-}$ control mice ($P < 0.01$) and from 8.2×10^6 to 4.2×10^6 i-IEL in $\beta 2m^{-/-}$ mutants ($P < 0.01$). Although we cannot explain this discrepancy, we consider two alternative explanations. It is possible that listerial invasion selectively

destroyed columnar epithelial cells, thus increasing the relative proportion of i-IEL in PAS-stained sections. Alternatively, it is possible that intestinal listeriosis rendered i-IEL more susceptible to the in vitro preparation procedure, thus causing their selective loss. In the next experiment, i-IEL were characterized phenotypically by flow cytometry. Consistent with previous data (26, 49), in $\beta 2m^{-/-}$ mutants CD8 α/β^+ TcR α/β i-IEL were absent (Table 2). Moreover, the CD8 α/α^+ TcR α/β i-IEL population was markedly reduced in $\beta 2m^{-/-}$ mutant mice (7, 26, 31, 35). Importantly, intestinal listeriosis had only negligible effects on the relative proportions of the various i-IEL populations, regardless of its marked influence on the biological activities of CD8 β^- i-IEL (Table 2). It is possible that the activation of these i-IEL during listerial invasion occurred virtually independently of i-IEL expansion. Alternatively, it is conceivable that intestinal listeriosis caused expansion of listeria-reactive i-IEL without affecting the overall homeostasis of the various i-IEL populations and that preactivated listeria-reactive i-IEL expressed more potent biological activities in response to TcR engagement.

DISCUSSION

L. monocytogenes is an intracellular pathogen which has been instrumental in elucidating the mechanisms of central cell-mediated antibacterial immunity (17). Although CD8 α/β^+ TcR α/β cells are essential for protective immunity, both TcR γ/δ cells and CD4 $^+$ TcR α/β cells contribute to optimum protection in addition (17). IFN- γ and target cell lysis are considered crucial for sterile clearance of the pathogen (17). Listeriosis is a relatively rare disease of humans, but food-borne outbreaks are being increasingly noted (8, 22, 34). It is reasonable to assume that mucosal immunity provides a first line of defense against listerial outbreaks. For this reason, we have started to analyze the role of i-IEL in mucosal protection, using the model of p.o. infection of mice with *L. monocytogenes*. Previous studies from our group have already suggested that intestinal listeriosis stimulates IFN- γ production in TcR γ/δ i-IEL of immunocompetent mice (47).

The vast majority (>80%) of all i-IEL express the CD8 coreceptor (14, 18, 20, 30). Approximately one-third of these CD8 $^+$ i-IEL possess the CD8 α/β heterodimer and about two-thirds possess the CD8 α/α homodimer (14, 18). While the

FIG. 1. Cytotoxic activities of i-IEL from $\beta 2m^{-/-}$ or $\beta 2m^{+/-}$ mutant mice and influence of intestinal *L. monocytogenes* infection. Mice were infected p.o. with 10^8 *L. monocytogenes* organisms. The i-IEL were prepared on day 0 or day 8 of infection and assayed for cytolytic activities against ^{51}Cr -labelled P815 cells in the presence of anti-CD3 MAb ($\blacklozenge$), anti-TcR α/β MAb ($\blacksquare$), anti-TcR γ/δ MAb ($\blacktriangle$), or normal hamster Ig ($\circ$) or in the absence of MAb ($\triangle$). Results were plotted as percent specific lysis at various effector-to-target (E:T) ratios. Representative results for a total of six mice per group are shown. *, significant difference relative to TcR α/β engagement at $P < 0.01$. ** and ***, significant difference relative to uninfected $\beta 2m^{-/-}$ mice at $P < 0.05$ and $P < 0.01$, respectively.

former population resides exclusively in the TcR α/β i-IEL population, the latter subset segregates into approximately equal proportions of TcR α/β and TcR γ/δ cells. The CD8 α/β^+ i-IEL are strictly $\beta 2m$ dependent, whereas the CD8 α/α^+ i-IEL are virtually $\beta 2m$ independent (7, 31, 35, 49). Therefore, $\beta 2m^{-/-}$ and $\beta 2m^{+/-}$ mutant mice allowed us to study the influences of listeriosis on the three major i-IEL populations, namely CD8 α/α^+ TcR α/β i-IEL, CD8 α/β^+ TcR α/β i-IEL, and CD8 α/α^+ TcR γ/δ i-IEL. We determined total cytolytic activities and frequencies of IFN- γ -producing cells following TcR engagement to assess the influence of intestinal listeriosis on i-IEL populations irrespective of their antigen specificity.

Consistent with previous studies (7, 26, 31, 35), flow cytometric analysis of i-IEL from $\beta 2m^{+/-}$ and $\beta 2m^{-/-}$ mice revealed not only a strict $\beta 2m$ dependence of CD8 α/β^+ i-IEL but also a strong influence on the CD8 α/α^+ TcR α/β i-IEL though not on the CD8 α/α^+ TcR γ/δ i-IEL. Complete loss of CD8 β^+ cells and marked diminution in the CD8 β^- population caused dramatic reduction of the total TcR α/β i-IEL population and a relative increase in the TcR γ/δ i-IEL population in $\beta 2m^{-/-}$ mice. Concomitantly, frequencies of IFN- γ producers as well

as cytolytic activities in response to TcR α/β ligation were reduced in these mutants. Yet, this reduction in biological activities was less pronounced, as could have been expected from flow cytometric analysis. These findings suggest that, at least in the absence of $\beta 2m$, the CD8 β^- i-IEL are potent IFN- γ producers and killer cells.

Mucosal host defense primarily involves production and transcytosis to the gut lumen of secretory IgA as well as regional activation of professional phagocytes, macrophages, and polymorphonuclear leukocytes (23). IFN- γ is a potent activator of professional phagocytes and, moreover, has been shown to promote expression of the IgA secretory component in epithelial cells (38). Furthermore, IFN- γ could induce MHC II expression in intestinal cells and thus promote antigen presentation by these cells to CD4 $^+$ T cells (40, 48). Finally, prompt IFN- γ secretion by i-IEL in response to intestinal listeriosis could contribute to a cytokine milieu which favors development of Th1 cells. This assumption is based on increasing evidence that the development of protective Th1 cells is markedly promoted by early IFN- γ secretion from other leukocytes (36). Although we consider IFN- γ secretion by i-IEL to be

FIG. 2. Kinetics of cytolytic activities and frequencies of IFN- γ -producing cells among i-IEL from $\beta 2m^{-/-}$ and $\beta 2m^{+/-}$ mice during intestinal *L. monocytogenes* infection. Mice were infected p.o. with 10^8 *L. monocytogenes* organisms, and i-IEL were prepared on day 0, 2, 4, or 8 postinfection. Cytotoxic activities of i-IEL (top graphs, effector-to-target ratio = 100:1) and frequencies of IFN- γ -producing cells among i-IEL (bottom graphs) were determined after stimulation with anti-CD3 MAb ($\blacklozenge$), anti-TcR α/β MAb ($\blacksquare$), anti-TcR γ/δ MAb ($\blacktriangle$), or normal hamster Ig ($\circ$). Results are means for a total of four mice per group. *, **, and ***, significant difference relative to uninfected mice at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. For further details, see Table 1 and the legend to Fig. 1.

important for mucosal defense against bacterial invasion from the intestine, we envisage that, in addition, cytolytic activities may contribute to mucosal resistance. In contrast to many other bacterial pathogens, invasion by *L. monocytogenes* is not restricted to M cells but seems to proceed through enterocytes as well (33). Lysis of infected enterocytes could interfere with bacterial invasion.

The $\beta 2m$ is required for surface expression of the classical MHC I α chain as well as of numerous "MHC I-like" molecules, including CD1 and TL (37, 41, 45). MHC I molecules present antigenic peptides to CD8 α/β^+ T cells, whereas the MHC I-like molecules serve as important presenting molecules for TcR γ/δ cells (15) and some TcR α/β cells, such as double-negative TcR α/β cells (4, 28, 29), CD4 $^+$ NK1.1 $^+$ TcR α/β cells

(5), and CD8 α/α^+ TcR α/β cells (1). The $\beta 2m$ -associated MHC I-like molecules of the CD1 family are expressed in high density by intestinal epithelial cells and present antigenic ligands to CD8 α/α^+ i-IEL (6, 27). Our experiments revealed a definite though not complete reduction of CD8 α/α^+ TcR α/β , but not TcR γ/δ , i-IEL in $\beta 2m^{-/-}$ mice. This may mean that the CD8 α/α^+ TcR α/β i-IEL encompasses MHC I-like-dependent and -independent subsets and that all TcR γ/δ i-IEL are independent. Our findings that intestinal listeriosis stimulated TcR α/β and TcR γ/δ i-IEL from $\beta 2m^{-/-}$ mutants exclude activation by $\beta 2m$ -associated classical MHC I and MHC I-like molecules, including CD1. Two alternative activation mechanisms are considered. First, some MHC I-like molecules are surface expressed independently of $\beta 2m$ and, therefore, could

TABLE 2. Phenotypes of i-IEL from $\beta 2m^{-/-}$ and $\beta 2m^{+/-}$ mice and influence of intestinal *L. monocytogenes* infection^a

Group ^b	Surface phenotype			% Lymphoid cells in mutant mice			
	CD4	CD8 α	CD8 β	Uninfected		Infected	
				$\beta 2m^{+/-}$	$\beta 2m^{-/-}$	$\beta 2m^{+/-}$	$\beta 2m^{-/-}$
A	—	—	—	3.4 $\pm$ 3.9	5.8 $\pm$ 4.8	8.4 $\pm$ 5.3	6.4 $\pm$ 4.9
B	+	—	—	4.3 $\pm$ 2.4	5.1 $\pm$ 1.8	7.5 $\pm$ 2.4	3.9 $\pm$ 1.8
C	+	+	—	4.4 $\pm$ 1.5	2.3 $\pm$ 1.8	2.2 $\pm$ 0.4	1.0 $\pm$ 0.3
D	+	+	+	ND ^c	ND	ND	ND
E	—	+	—	21.0 $\pm$ 8.4	0.5 $\pm$ 0.1 ^d	14.7 $\pm$ 4.1	2.1 $\pm$ 1.2
F	—	+	+	25.3 $\pm$ 8.1	ND ^d	19.5 $\pm$ 4.7	ND
G	—	—	—	0.5 $\pm$ 0.3	1.2 $\pm$ 0.5	0.8 $\pm$ 0.5	1.1 $\pm$ 0.9
H	+	—	—	ND	ND	ND	ND
I	+	+	—	ND	ND	ND	ND
J	+	+	+	ND	ND	ND	ND
K	—	+	—	30.4 $\pm$ 14.3	67.7 $\pm$ 12.1 ^e	33.9 $\pm$ 4.1	70.2 $\pm$ 14.3
L	—	+	+	ND	ND	ND	ND
M	—	—	—	4.0 $\pm$ 4.1	6.8 $\pm$ 3.9	4.9 $\pm$ 0.9	5.6 $\pm$ 2.1

^a Mice were infected p.o. with 10^8 *L. monocytogenes* organisms. The i-IEL were prepared on day 0 and day 8 postinfection and double or triple stained. Data from three different experiments, means $\pm$ standard deviations for 10 mice, are shown.

^b Groups: A, B220⁺ cells; B, CD4⁺ CD8 α ⁺ TcR α / β cells; C, (CD4⁺ CD8 α ⁺ TcR α / β cells) – (CD4⁺ CD8 β ⁺ TcR α / β cells); D, CD4⁺ CD8 β ⁺ TcR α / β cells; E, (CD4⁺ CD8 α ⁺ TcR α / β cells) – (CD4⁺ CD8 β ⁺ TcR α / β cells); F, CD4⁺ CD8 β ⁺ TcR α / β cells; G, CD4⁺ CD8 α ⁺ TcR α / β cells; H, CD4⁺ CD8 α ⁺ TcR γ / δ cells; I, (CD4⁺ CD8 α ⁺ TcR γ / δ cells) – (CD4⁺ CD8 β ⁺ TcR γ / δ cells); J, CD4⁺ CD8 β ⁺ TcR γ / δ cells; K, (CD4⁺ CD8 α ⁺ TcR γ / δ cells) – (CD4⁺ CD8 β ⁺ TcR γ / δ cells); L, CD4⁺ CD8 β ⁺ TcR γ / δ cells; M, CD4⁺ CD8 α ⁺ TcR γ / δ cells.

^c ND, not detectable.

^d Significant difference relative to uninfected $\beta 2m^{+/-}$ mice at $P < 0.001$.

^e Significant difference relative to uninfected $\beta 2m^{+/-}$ mice at $P < 0.01$.

participate in the activation of i-IEL in $\beta 2m^{-/-}$ mice. Alternatively, these i-IEL could be activated independently of TcR-ligand interactions in vivo, e.g., through inflammatory cytokines. Because of the rapid activation of i-IEL in $\beta 2m^{-/-}$ mice, we favor the latter alternative.

Surface expression of neonatal Fc receptor also depends on $\beta 2m$ association (37). The neonatal Fc receptor participates in IgA transcytosis into the gut lumen (37). Although we consider the possibility that deficient neonatal Fc receptor expression affects mucosal protection in $\beta 2m^{-/-}$ mice, we have thus far no evidence for increased translocation of normal gut flora nor for increased susceptibility to intestinal listeriosis in $\beta 2m^{-/-}$ mutants (unpublished data). Careful studies on this subject will be performed in the future.

The i-IEL express potent cytolytic activities and synthesize various cytokines directly in response to TcR ligation without intentional preactivation (10, 42, 44). Such preactivation is probably caused by continuous encounter with the normal intestinal flora (2). Our experiments, showing potent cytolytic activity and IFN- γ production by i-IEL both in $\beta 2m^{+/-}$ and in $\beta 2m^{-/-}$ mice, are consistent with preactivation of i-IEL by the normal gut flora independent of $\beta 2m$ expression. Yet, our data reveal further elevation of biological activities by TcR γ / δ i-IEL at an inoculum dose which results in host invasion from the intestine (47). Thus, the i-IEL were not exhausted and could be stimulated further by invasive pathogens.

Frequencies of IFN- γ -producing TcR γ / δ i-IEL were low in uninfected $\beta 2m^{+/-}$ and $\beta 2m^{-/-}$ mice compared with those of TcR α / β i-IEL. In response to listeriosis, frequencies of IFN- γ -producing TcR γ / δ i-IEL were increased 2- to 3-fold in $\beta 2m^{+/-}$ mice and 10-fold in $\beta 2m^{-/-}$ mice. This differential activation of TcR γ / δ i-IEL in $\beta 2m^{+/-}$ and $\beta 2m^{-/-}$ mice could mean that TcR γ / δ i-IEL are inhibited by $\beta 2m$. Evidence that TcR γ / δ i-IEL encompass a large fraction of autoreactive T cells which are controlled locally in the intestine has been presented (25). Recently, inhibitory receptors which interact with conserved parts of the MHC I molecule have been identified on activated NK cells and on cytotoxic T lymphocytes (3).

It is possible that such inhibitory mechanisms prevent uncontrolled activation of TcR γ / δ i-IEL in immunocompetent mice. Obviously, in the absence of $\beta 2m$, such inhibitory signals cannot be transmitted, thus causing potent activation of TcR γ / δ i-IEL to intestinal listeriosis in $\beta 2m^{-/-}$ mice.

In summary, our findings demonstrate that intestinal listeriosis increases biological activities in i-IEL, in particular IFN- γ production in the presence or in the absence of $\beta 2m$. Hence, CD8 α / α ⁺ TcR γ / δ i-IEL and a large proportion of CD8 α / α ⁺ TcR α / β i-IEL not only develop independently of $\beta 2m$, but their functional activation by invading bacterial pathogens also occurs independently of $\beta 2m$. Rapid activation of these i-IEL could contribute to immune surveillance against bacterial invasion at the mucosal barrier.

ACKNOWLEDGMENTS

We are grateful to R. Jaenisch for breeding pairs of mutant mice and to L. Lefrançois, R. Kubo, J. A. Bluestone, and M. Pierres for helpful reagents. We thank K. Tell for MAb production and R. Mahmoudi and A. Gritzan for excellent secretarial assistance.

This work received financial support from the Deutsche Forschungsgemeinschaft (SFB 322 and Ka 573/3-1). M. Emoto acknowledges financial support from the Nitto Foundation and the Mochida Memorial Foundation for Medical and Pharmaceutical Research.

REFERENCES

- Balk, S. P., E. C. Ebert, R. L. Blumenthal, F. V. McDermott, K. W. Wucherpfennig, S. B. Landau, and R. S. Blumberg. 1991. Oligoclonal expansion and CD1 recognition by human intestinal intraepithelial lymphocytes. *Science* 253:1411–1415.
- Bandeira, A., T. Mota-Santos, S. Itoharu, S. Degermann, C. Heusser, S. Tonegawa, and A. Coutinho. 1990. Localization of $\gamma\delta$ T cells to the intestinal epithelium is independent of normal microbial colonization. *J. Exp. Med.* 172:239–244.
- Barinaga, M. 1995. Researchers find molecules that muzzle killer cells. *Science* 268:367–368.
- Beckman, E. M., S. A. Porcelli, C. T. Morita, S. M. Behar, S. T. Furlong, and M. B. Brenner. 1994. Recognition of a lipid antigen by CD1-restricted $\alpha\beta$ ⁺ T cells. *Nature (London)* 372:691–694.
- Bendelac, A., O. Lantz, M. E. Quimby, J. W. Yewdell, J. R. Bennink, and

- R. R. Brutkiewicz. 1995. CD1 recognition by mouse NK1⁺ T lymphocytes. *Science* **268**:863–865.
6. Bleicher, P. A., S. P. Balk, S. J. Hagen, R. S. Blumberg, T. J. Flotte, and C. Terhorst. 1990. Expression of murine CD1 on gastrointestinal epithelium. *Science* **250**:679–682.
7. Correa, L., M. Bix, N.-S. Liao, M. Zijlstra, R. Jaenisch, and D. Raulet. 1992. Most γ/δ T cells develop normally in $\beta 2$ -microglobulin-deficient mice. *Proc. Natl. Acad. Sci. USA* **89**:653–657.
8. Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brondum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *N. Engl. J. Med.* **312**:404–407.
9. Golstein, P., C. Goridis, A.-M. Schmitt-Verhulst, B. Hayot, A. Pierres, A. van Aghoven, Y. Kaufmann, Z. Eshhar, and M. Pierres. 1982. Lymphoid cell surface interaction structures detected using cytolysis-inhibiting monoclonal antibodies. *Immunol. Rev.* **68**:5–42.
10. Goodman, T., and L. Lefrançois. 1988. Expression of the γ/δ T-cell receptor on intestinal CD8⁺ intraepithelial lymphocytes. *Nature (London)* **333**:855–858.
11. Goodman, T., and L. Lefrançois. 1989. Intraepithelial lymphocytes. Anatomical site, not T cell receptor form, dictates phenotype and function. *J. Exp. Med.* **170**:1569–1581.
12. Guy-Grand, D., M. Malassis-Seris, C. Briottet, and P. Vassalli. 1991. Cytotoxic differentiation of mouse gut thymodependent and independent intraepithelial T lymphocytes is induced locally. Correlation between functional assays, presence of perforin and granzyme transcripts, and cytoplasmic granules. *J. Exp. Med.* **173**:1549–1552.
13. Guy-Grand, D., B. Rocha, and P. Vassalli. 1993. Origin and development of gut intraepithelial lymphocytes, p. 21–31. *In* H. Kiyono and J. R. McGhee (ed.), *Advances in host defense mechanisms*, vol. 9. Mucosal immunology: intraepithelial lymphocytes. Raven Press Ltd., New York.
14. Guy-Grand, D., and P. Vassalli. 1993. Gut intraepithelial T lymphocytes. *Curr. Opin. Immunol.* **5**:247–252.
15. Haas, W., P. Pereira, and S. Tonegawa. 1993. Gamma/delta cells. *Annu. Rev. Immunol.* **11**:637–685.
16. Ishikawa, H., Y. Li, A. Abeliovich, S. Yamamoto, S. H. E. Kaufmann, and S. Tonegawa. 1993. Cytotoxic and interferon γ -producing activities of γ/δ T cells in the mouse intestinal epithelium are strain dependent. *Proc. Natl. Acad. Sci. USA* **90**:8204–8208.
17. Kaufmann, S. H. E. 1993. Immunity to intracellular bacteria. *Annu. Rev. Immunol.* **11**:129–163.
18. Klein, J. R., and R. L. Mosley. 1993. Phenotypic and cytotoxic characteristics of intraepithelial lymphocytes, p. 33–60. *In* H. Kiyono and J. R. McGhee (ed.), *Advances in host defense mechanisms*, vol. 9. Mucosal immunology: intraepithelial lymphocytes. Raven Press Ltd., New York.
19. Kubo, R. T., W. Born, J. W. Kappler, P. Marrack, and M. Pigeon. 1989. Characterization of a monoclonal antibody which detects all murine $\alpha\beta$ T cell receptors. *J. Immunol.* **142**:2736–2742.
20. Lefrançois, L. 1991. Extrathymic differentiation of intraepithelial lymphocytes: generation of a separate and unequal T-cell repertoire? *Immunol. Today* **12**:436–438.
21. Leo, O., M. Foo, D. H. Sachs, L. E. Samelson, and J. A. Bluestone. 1987. Identification of a monoclonal antibody specific for a murine T3 polypeptide. *Proc. Natl. Acad. Sci. USA* **84**:1374–1378.
22. Linnan, M. J., L. Mascola, X. D. Lou, V. Goulet, S. May, C. Salminen, D. W. Hird, M. L. Yonekura, P. Hayes, R. Weaver, A. Audurier, B. D. Plikaytis, S. L. Fannin, A. Kleks, and C. V. Broome. 1988. Epidemic listeriosis associated with Mexican-style cheese. *N. Engl. J. Med.* **319**:823–828.
23. Mestecky, J., and J. R. McGhee. 1987. Immunoglobulin A (IgA): molecular and cellular interactions involved in IgA biosynthesis and immune response. *Adv. Immunol.* **40**:153–245.
24. Möller, S. A., and C. A. Borrebaeck. 1985. A filter immuno-plaque assay for the detection of antibody-secreting cells in vitro. *J. Immunol. Methods* **79**:195–204.
25. Nagler-Anderson, C., L. A. McNair, and A. Cradock. 1992. Self-reactive, T cell receptor- $\gamma\delta$ ⁺, lymphocytes from the intestinal epithelium of weanling mice. *J. Immunol.* **149**:2315–2322.
26. Neuhaus, O., M. Emoto, C. Blum, S. Yamamoto, and S. H. E. Kaufmann. 1995. Control of thymus-independent intestinal intraepithelial lymphocytes by $\beta 2$ -microglobulin. *Eur. J. Immunol.* **25**:2332–2339.
27. Porcelli, S. A. 1995. The CD1 family: a third lineage of antigen-presenting molecules. *Adv. Immunol.* **59**:1–98.
28. Porcelli, S., M. B. Brenner, J. L. Greenstein, S. P. Balk, C. Terhorst, and P. A. Bleicher. 1989. Recognition of cluster of differentiation 1 antigens by human CD4⁺CD8⁺ cytolytic T lymphocytes. *Nature (London)* **341**:447–450.
29. Porcelli, S., C. T. Morita, and M. B. Brenner. 1992. CD1b restricts the response of human CD4⁺8⁺ T lymphocytes to a microbial antigen. *Nature (London)* **360**:593–597.
30. Poussier, P., and M. Julius. 1993. Maturation of T cells in the intestinal epithelium. *Immunol. Today* **14**:140.
31. Poussier, P., and M. Julius. 1994. Thymus independent T cell development and selection in the intestinal epithelium. *Annu. Rev. Immunol.* **12**:521–553.
32. Prat, M., G. Gribaudo, P. M. Comoglio, G. Cavallo, and S. Landolfo. 1984. Monoclonal antibodies against murine γ interferon. *Proc. Natl. Acad. Sci. USA* **81**:4515–4519.
33. Racz, P., K. Tenner, and E. Mero. 1972. Experimental listeria enteritis. I. An electron microscopic study of the epithelial phase in experimental listeria infection. *Lab. Invest.* **26**:694–700.
34. Schlech, W. F., P. M. Lavigne, R. A. Bortolussi, A. C. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nicholls, and C. V. Broome. 1983. Epidemic listeriosis—evidence for transmission by food. *N. Engl. J. Med.* **308**:203–206.
35. Schlessner, C., and R. Ceredig. 1993. Analysis of intraepithelial lymphocytes from major histocompatibility complex (MHC)-deficient mice: no evidence for a role of MHC class II antigens in the positive selection of V δ ⁺ γ/δ T cells. *Eur. J. Immunol.* **23**:1615–1622.
36. Seder, R. A., and W. E. Paul. 1994. Acquisition of lymphokine-producing phenotype by CD4⁺ T cells. *Annu. Rev. Immunol.* **12**:635–673.
37. Shawar, S. M., J. M. Vyas, J. R. Rodgers, and R. R. Rich. 1994. Antigen presentation by major histocompatibility complex class I-b molecules. *Annu. Rev. Immunol.* **12**:839–880.
38. Solld, L. M., D. Kvale, P. Brandtzaeg, G. Markussen, and E. Thorsby. 1987. Interferon- γ enhances expression of secretory component, the epithelial receptor for polymeric immunoglobulins. *J. Immunol.* **138**:4303–4306.
39. Spitalny, G. L., and E. A. Havell. 1984. Monoclonal antibody to murine gamma interferon inhibits lymphokine-induced antiviral and macrophage tumoricidal activities. *J. Exp. Med.* **159**:1560–1565.
40. Steiniger, B., P. Falk, M. Lohmüller, and P. H. van der Meide. 1989. Class II MHC antigens in the rat digestive system. Normal distribution and induced expression after interferon-gamma treatment *in vivo*. *Immunology* **68**:507–513.
41. Stroynowski, I. 1990. Molecules related to class I major histocompatibility complex antigens. *Annu. Rev. Immunol.* **8**:501–530.
42. Taguchi, T., J. R. McGhee, R. L. Coffman, K. W. Beagley, J. H. Eldridge, K. Takatsu, and H. Kiyono. 1990. Analysis of Th1 and Th2 cells in murine gut-associated tissues. Frequencies of CD4⁺ and CD8⁺ T cells that secrete IFN- γ and IL-5. *J. Immunol.* **145**:68–77.
43. Teixeira, H. C., and S. H. E. Kaufmann. 1994. Role of NK1.1⁺ cells in experimental listeriosis. NK1⁺ cells are early IFN- γ producers but impair resistance to *Listeria monocytogenes* infection. *J. Immunol.* **152**:1873–1882.
44. Viney, J. L., P. J. Kilshaw, and T. T. MacDonald. 1990. Cytotoxic $\alpha\beta$ ⁺ and $\gamma\delta$ ⁺ T cells in murine intestinal epithelium. *Eur. J. Immunol.* **20**:1623–1626.
45. von Boehmer, H. 1990. Developmental biology of T cells in T cell-receptor transgenic mice. *Annu. Rev. Immunol.* **8**:531–556.
46. von Boehmer, H., W. Swat, and P. Kieselow. 1993. Positive selection of immature $\alpha\beta$ T cells. *Immunol. Rev.* **135**:67–79.
47. Yamamoto, S., F. Russ, H. C. Teixeira, P. Conradt, and S. H. E. Kaufmann. 1993. *Listeria monocytogenes*-induced gamma interferon secretion by intestinal intraepithelial γ/δ T lymphocytes. *Infect. Immun.* **61**:2154–2161.
48. Zhang, Z., and J. G. Michael. 1990. Orally inducible immune unresponsiveness is abrogated by IFN- γ treatment. *J. Immunol.* **144**:4163–4165.
49. Zijlstra, M., M. Bix, N. E. Simister, J. M. Loring, D. H. Raulet, and R. Jaenisch. 1990. $\beta 2$ -microglobulin deficient mice lack CD4⁺8⁺ cytolytic T cells. *Nature (London)* **344**:742–746.