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Expression of Mouse Interleukin-4 by a Recombinant Ectromelia Virus Suppresses Cytolytic Lymphocyte Responses and Overcomes Genetic Resistance to Mousepox

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Genetic resistance to clinical mousepox (ectromelia virus) varies among inbred laboratory mice and is characterized by an effective natural killer (NK) response and the early onset of a strong $CD8^+$ cytotoxic T-lymphocyte (CTL) response in resistant mice. We have investigated the influence of virus-expressed mouse interleukin-4 (IL-4) on the cell-mediated response during infection. It was observed that expression of IL-4 by a thymidine kinase-positive ectromelia virus suppressed cytolytic responses of NK and CTL and the expression of gamma interferon by the latter. Genetically resistant mice infected with the IL-4-expressing virus developed symptoms of acute mousepox accompanied by high mortality, similar to the disease seen when genetically sensitive mice are infected with the virulent Moscow strain. Strikingly, infection of recently immunized genetically resistant mice with the virus expressing IL-4 also resulted in significant mortality due to fulminant mousepox. These data therefore suggest that virus-encoded IL-4 not only suppresses primary antiviral cell-mediated immune responses but also can inhibit the expression of immune memory responses.

Ectromelia virus (ECTV; family *Poxviridae*, genus *Orthopoxvirus*) is a natural pathogen of laboratory mice that causes a generalized disease termed mousepox (13). All mice are equally susceptible to infection by footpad inoculation; however, development of clinical mousepox among inbred mouse strains differs greatly (44). In mousepox-sensitive (e.g., BALB/c) mice, the disease is an acute systemic infection with high viral titers in the liver and spleen with resultant necrosis and high mortality. In contrast, infection of mousepox-resistant (e.g., C57BL/6) mice is usually subclinical, with lower levels of viral replication in the visceral organs and development of nonfatal lesions. Genetic resistance has been found to act through the combined activity of innate host defenses including natural killer (NK) cells, alpha interferon (IFN-α), IFN-β, IFN-γ, activated macrophages, and inducible nitric oxide production (17, 21, 23, 24, 36). Mousepox-resistant mice also display the early activation of a strong virus-specific cytotoxic T-lymphocyte (CTL) response (20, 32) and produce high levels of type 1 cytokines interleukin-2 (IL-2), IL-12, IFN-γ, and tumor necrosis factor alpha (TNF-α) in response to ECTV infection, whereas these factors are absent or produced at low levels in susceptible mice (19, 36).

Effector $CD4^+$ T cells can be categorized on the basis of their cytokine production either as T helper 1 (Th1) cells that produce IL-2 and IFN-γ or T helper 2 (Th2) cells that produce predominantly IL-4, IL-5, IL-10, and IL-13 (40). The cross-regulatory activities of IL-12 and IL-4, factors that play key roles in directing the development of the Th1 and Th2 subsets, respectively, is well characterized (40). Both in vitro and in vivo, the presence of IL-4 at the time of stimulation has been shown to inhibit IL-12 expression by antigen-presenting cells (macrophages and dendritic cells), with Th2 cells dominantly expanded in the acquired response (7, 8, 25). In addition to its effects on development of Th1 and Th2 subsets, IL-4 has been shown to influence the differentiation of other lymphocyte types. In vitro stimulation of naive $CD8^+$ cells in the presence of IL-2, IL-12, or IFN-γ generates classical type 1 cytotoxic cells (Tc1) which express IFN-γ; however, $CD8^+$ cells stimulated in the presence of IL-4 may develop a Tc2 phenotype expressing the cytokines IL-4, IL-5, IL-6, IL-10, sometimes with reduced cytoxicity (11, 38). Treatment of activated Tc1 cells with IL-4 results in defective IFN-γ, TNF-α, and IL-2 expression. Although IL-4-treated Tc1 cells retain short-term in vitro cytotoxic activity, they fail to proliferate in response to antigen stimulation, compromising their long-term functional capability to control infection (37). It has recently been shown that NK cells cultured in the presence of IL-12 or IL-4 may also differentiate into NK1 or NK2 cells, respectively, with distinct patterns of cytokine secretion similar to those of Th1 and Th2 cells, although this does not appear to affect their in vitro cytotoxic activity (33).

Cross-regulation of Th subsets and the generation of an appropriate type of immune response against a particular pathogen is important since the dominance of an inappropriate response can exacerbate disease and lead to the inability to eradicate the infecting organism. The use of recombinant vaccinia virus (VACV) to study the in vivo effects of mouse cytokines has demonstrated that the course of infection can be mediated and biased toward either an antiviral effect by coexpression of type 1 cytokines or enhanced virus virulence by

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coexpression of selected type 2 cytokines (36, 41). Previous studies using a variety of viral infection models have shown that overexpression or systemic administration of IL-4 impedes the development of virus-specific CTL activity, causing a delay in viral clearance, although infected mice generally survive infection (2, 14, 27, 41). Furthermore, VACV-expressed IL-4 inhibits the expression of type 1 cytokines (41), biasing the antibody response in favor of a Th2-mediated immunoglobulin G1 profile (2).

Infection of mice with non-mouse-adapted VACV can be controlled in the absence of CTL activity in $CD8^+$ T-cell-deficient or -depleted mice (36, 42). It has been proposed that control of VACV and other cytopathic virus infections is accomplished by compensating antiviral soluble mediators such as IFN-γ, TNF-α, and possibly antibody, and that the lytic activity of $CD8^+$ cells plays only a peripheral role (18, 35). Although the orthopoxviruses VACV and ECTV are both cytopathic viruses and are very closely related at the genetic level, they differ greatly in pathogenicity in the mouse. Only in immunocompromised mice does VACV cause a severe disease following infection with relatively high doses of virus. In contrast, infection with low doses of ECTV is sufficient to generate clinical disease in most mouse strains (36, 44). This is because ECTV is naturally mouse adapted and expresses virulence factors, such as the IFN-γ binding protein (28), that have greater affinity for the mouse than the VACV equivalents. It has been established that macrophages (20, 43), NK cells (17), and $CD8^+$ T cells (20) are all crucial for controlling infection with ECTV. IFN-γ- or IFN-γ receptor-deficient mice are also highly susceptible to mousepox even in the presence of normal CTL activity (19, 23, 36). Thus, the neutralization of any one of the major innate or adaptive cell-mediated antiviral activities has been shown to result in fulminant mousepox in otherwise genetically resistant mice. The study of coexpressed mouse cytokines in the context of a pathogenic host-adapted viral vector provides the opportunity to study immunological function in a situation where the cell-mediated immune response is crucial for recovery.

In the course of our studies into the development of virally vectored immunocontraceptive vaccines (16), we have investigated the ability of coexpressed type 2 cytokines to act as adjuvants to enhance an antibody-mediated response. We have observed that thymidine kinase (TK)-positive recombinant ECTVs expressing mouse IL-4 are highly virulent and that infection of mice with these viruses suppresses NK and CTL cytolytic activity and IFN-γ expression by splenic $CD8^+$ T cells. Suppression of cellular immunity in both mousepox-resistant mice and ECTV-immune mice resulted in acute mousepox with a high mortality rate.

MATERIALS AND METHODS

Cells and viruses. L-M(TK−) (mouse [*Mus musculus*] ATCC CCL-1.3) and B-SC-1 (African green monkey [*Cercopithecus aethiops*] ATCC CCL-26) cells were maintained in minimal essential medium (MEM) supplemented with 5% fetal bovine serum at 37°C in 5% CO_2. ECTVs were grown on the above cells in MEM at 35°C in 5% CO_2. Recombinant ECTVs were constructed by infection of L-M(TK−) cells at a multiplicity of infection of 0.1 PFU/cell with the TK^- virus ECTV-602, which contains an insertion of the *Escherichia coli lacZ* (β-galactosidase) gene inactivating the orthopoxvirus TK gene (16). The virus-infected cells were transfected with plasmid pTK-7.5A (5) or pFB-TK-IL4 (1), using LipofectAMINE reagent (Gibco-BRL Life Technologies Inc., Gaithersburg, Md.). With these vectors, DNA recombination should occur between the VACV *Hin*dIII-F sequences contained in the vectors and the homologous sequences within ECTV *Hin*dIII-E. Recombinant ECTVs expressing the herpes simplex virus (HSV) TK gene were selected by growth on L-M(TK−) cells in MEM containing hypoxanthine-aminopterin-thymidine supplement (Gibco-BRL Life Technologies). Recombinant virus ECTV-602(TK+), which was constructed using pTK-7.5A, contains a copy of the HSV TK gene inserted into the natural *Bam*HI site located immediately downstream of the of the VACV F7 open reading frame early promoter. Virus ECTV-IL4(TK+), which was constructed using pFB-TK-IL4, is similar to ECTV-602(TK+) except that it also contains a copy of the mouse IL-4 cDNA under the transcriptional control of the VACV P7.5 early/late promoter inserted immediately downstream of the HSV TK gene. IL-4 expression was confirmed in vitro by bioassay of supernatants from ECTV-IL4(TK+)-infected L-M(TK−) cell cultures (15, 41).

Virus titration. Titration of recombinant ECTVs recovered from mouse tissues was performed on B-SC-1 cells grown in six-well culture dishes overlaid with 2 ml of MEM containing 1% (wt/vol) low-melting-point agarose (SeaPlaque GTG; FMC BioProducts, Rockland, Maine) and grown at 35°C for 72 h. Plaques produced by viruses expressing β-galactosidase were visualized by overlaying the infected cells with a further 2 ml of MEM–1% agarose containing X-Gal (5′-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; 300 μg/ml).

Mice and inoculation. Animal studies were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Specific-pathogen-free 6- to 8-week-old female mice were obtained from the Australian National University Animals Services Division.

(i) Virulence studies. BALB/c and C57BL/6 mice were inoculated with various amounts of virus into the right hind footpad, and disease symptoms were observed for 2 weeks postinfection when surviving animals were euthanized.

(ii) DTH studies. BALB/c and C57BL/6 mice were immunized by inoculation into the right hind footpad with 10^3 PFU of highly attenuated ECTV-602. The immune mice were challenged 4 weeks postimmunization by inoculation into the same footpad with 10^4 PFU of either ECTV-602(TK+) or ECTV-IL4(TK+). Delayed-type hypersensitivity (DTH) responses were measured 24 and 48 h postchallenge by measuring the dorsoventral thickness of the inoculated right foot using calipers and compared to the thickness of the uninoculated left foot. Mice were monitored for a further 3 weeks to observed signs of disease and mortality.

(iii) Assessment of antiviral cytolytic responses and IFN-γ production. Female C57BL/6 mice were infected by footpad inoculation with 10^2, 10^3, or 10^4 PFU for CTL assays, or 10^4 PFU for NK cell assays or assessment of IFN-γ production, of either ECTV-602(TK+) or ECTV-IL4(TK+). For NK cell assays, spleens were removed on days 1, 2, and 3 postinfection (p.i.); for assays of CTL activity or IFN-γ expression, spleens were removed on day 7 p.i. NK cell cytolytic activity was measured on YAC-1 cells (ATCC TIB-160), while CTL activity was measured on VACV-infected MC57G targets (ATCC CRL-295; a C57BL/6 derived fibroblast line; $H\text{-}2^b$) using the standard 6-h ^{51}Cr release assay (22). IFN-γ production was measured using microcultures set up in parallel with those used in CTL assays. Splenic effector cells were cultured with VACV-infected or uninfected MC57G targets at a ratio of 20:1. Supernatants were collected after 6 h, and IFN-γ levels assayed by enzyme-linked immunosorbent assay (15).

RESULTS

Disease symptoms following infection of mice with recombinant ECTVs. (i) Control virus. Footpad inoculation of mousepox-resistant C57BL/6 mice with 10^3 PFU ECTV-602(TK+) caused symptoms similar to the those caused by wild-type Moscow strain of ECTV. However, unlike infection with the Moscow strain, recovery was not normally associated with necrosis and sloughing of the infected limb. In mousepox-sensitive BALB/c mice, ECTV-602(TK+) was clearly less virulent than the Moscow strain since it did not cause mortality and behaved similarly to infection of C57BL/6 mice. However, footpad inoculation of the highly sensitive A/J strain mice with ECTV-602(TK+) was generally lethal (data not shown). This is consistent with the known 100-fold-lower 50% lethal dose of the A/J strain relative to BALB/c mice following infection with the Moscow strain (13,31).

The vectors used here to construct the recombinant ECTVs

TABLE 1. Virus titers, in spleens of C57BL/6 mice 7 days after infection with recombinant control ECTV or ECTV expressing IL-4[a]

Virus	Titer (PFU/g) in spleen		
	10^2 PFU	10^3 PFU	10^4 PFU
Control ECTV	3.0×10^4	2.8×10^4	1.5×10^2
ECTV-602(TK+)			
ECTV-IL4(TK+)	2.5×10^5	3.5×10^7	6.6×10^7

[a] Mice were infected by footpad inoculation with the specified viral dose in a total volume of 10 μl.

utilize an insertion point immediately downstream of the early promoter (PF) for the VACV F7L open reading frame, which encodes a product of unknown function. This site is within a highly conserved region of the orthopoxvirus genome, and the open reading frame organization surrounding the insertion site is conserved in all orthopoxviruses sequenced to date. Use of these vectors will therefore disrupt expression of the F7L gene homologue and introduce VACV DNA sequences between the sites of recombination with the ECTV genome. The inserted HSV TK gene under the transcriptional control of the PF promoter has also been demonstrated to express only 10% of the endogenous orthopoxvirus TK activity (6). The combination of these factors generates recombinant ECTVs of intermediate pathogenicity between highly attenuated TK^- (16) and virulent wild-type Moscow viruses.

(ii) TK^+ IL-4-expressing virus. To assess the effects of IL-4 expression by a recombinant ECTV on virulence, we infected mice with 10^3 PFU of ECTV-IL4(TK+) and monitored disease symptoms and mortality. Infection of BALB/c or C57BL/6 mice with ECTV-IL4(TK+) proved to be uniformly lethal, with mean survival times (days) being 7.4 ± 0.7 ($n = 10/10$) and 8.6 ± 1.2 ($n = 10/10$), respectively. In both strains, swelling of the inoculated foot was clearly visible by 6 day p.i., and the foot continued to swell until the mice succumbed to the infection. Shortly before death, the infected mice became lethargic with ruffled fur and hunched posture. At autopsy, the mice contained enlarged pallid spleens and livers, with both organs containing numerous discrete white spots typical of necrotic lesions. These clinical signs and lesions are typical of acute mousepox as seen in genetically susceptible mice infected with the virulent Moscow strain (13). C57BL/6 mice infected with ECTV-IL4(TK+) contained high levels of virus in the spleen just prior to displaying early symptoms of acute mousepox (Table 1). In contrast, virus titers in the spleens of mice infected with equivalent doses of ECTV-602(TK+) were reduced, suggestive of immune mediated clearance. Increasing the infectious dose of the IL-4-expressing virus exacerbated the onset of symptoms and decreased survival times (data not shown). All control mice infected with equivalent doses of ECTV-602(TK+) survived infection.

(iii) CTL and NK responses and IFN-γ production following infection. Due to the enhanced virulence of the recombinant ECTV, we next assessed the effects of IL-4 expression on the development of antiviral $CD8^+$ CTL, NK activity, and IFN-γ expression, responses which are crucial for controlling ECTV infection. Increasing doses of ECTV-IL4(TK+) were used to infect C57BL/6 mice; spleens were isolated 7 days p.i. and assayed for CTL activity using the standard ^{51}Cr release assay (Fig. 1A). Splenocytes isolated from mice infected with the control virus ECTV-602(TK+) displayed significant specific cytolytic activity toward orthopoxvirus-infected MC57G cells. In contrast, splenocytes isolated from C57BL/6 mice infected with ECTV-IL4(TK+) contained no detectable virus-specific cytolytic activity.

IL-4 expression in ECTV also markedly suppressed IFN-γ secretion by antiviral $CD8^+$ T cells. Mean levels of virus-specific IFN-γ produced by splenocytes from mice infected with 10^4 PFU of ECTV-602(TK+) were sevenfold higher than background levels (733 ± 39 compared to 129 ± 29 U/ml). In contrast, $CD8^+$ T cells from mice given ECTV-IL4(TK+) failed to produce IFN-γ at levels above background (130 ± 47 U/ml).

To assay for induction of innate NK cell activity, spleen cells were isolated from ECTV-IL4(TK+)-infected mice on days 1, 2, and 3 p.i. and assayed for lytic activity on YAC-1 cells.

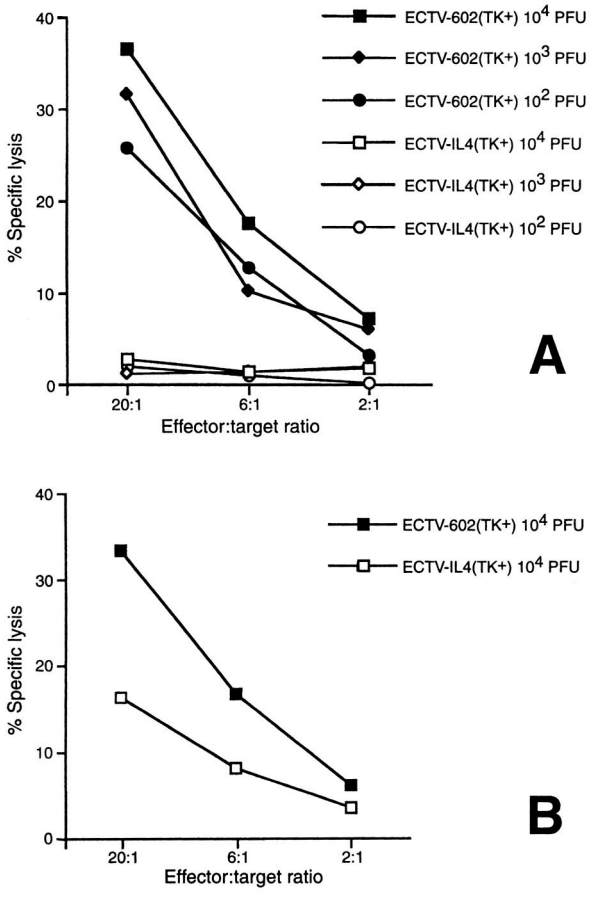

FIG. 1. (A) Antiorthopoxvirus CTL responses in C57BL/6 mice following footpad inoculation with various doses of ECTV-IL4(TK+) or ECTV-602(TK+). Lytic activity of splenocytes taken 7 days p.i. was determined at different effector-to-target ratios. Data shown are representative of two assays carried out in triplicate with four mice per group. Standard errors of the means at all points were <5%. (B) NK cell response in C57BL/6 mice following footpad inoculation with 10^4 PFU of ECTV-IL4(TK+) or ECTV-602(TK+) 3 days p.i. Lytic activities at different effector-to-target ratios are shown. Data shown are representative of two assays carried out in triplicate with four mice per group. Standard errors of the means at all points were <5%.

TABLE 2. Response of ECTV-immune mice to reinfection with ECTV-IL4(TK+)

Mouse strain	DTH response (0.1-mm units; mean ± SEM)[a]				% Mortality 6–8 days p.i. (no. dead/group of 5)	
	24 h p.i.		48 h p.i.			
	ECTV-602(TK+)	ECTV-IL4(TK+)	ECTV-602(TK+)	ECTV-IL4(TK+)	ECTV-602(TK+)	ECTV-IL4(TK+)
C57BL/6	4.4 ± 0.4	9.4 ± 0.5	2.2 ± 0.4	12.0 ± 0.5	0 (0)	60 (3)
BALB/c	4.8 ± 0.7	12.0 ± 0.3	2.0 ± 0.3	12.1 ± 0.8	0 (0)	60 (3)

[a] Difference in thickness of the inoculated footpad (10^4 PFU of virus) compared to the uninoculated foot.

Cytolytic activity was not detected at day 1 after infection with either control or IL-4-expressing viruses. At day 2 p.i., both groups of mice expressed approximately equivalent levels of NK activity (20% lysis of YAC-1 targets at 20:1 effector/target ratio). However, by day 3 p.i., when NK cell activity is usually near maximal during ECTV infection (29), ECTV-IL4(TK+)-infected C57BL/6 mice displayed approximately a threefold reduction in splenic NK cell-mediated lysis of YAC-1 targets compared to similarly infected controls [(Fig. 1B); compare percent specific lysis of ECTV-IL4(TK+)-infected mice at 20:1 effector/target ratio and control virus-infected mice at 6:1 effector/target ratio].

Reinfection of mice immunized against ECTV. Due to the observed suppression of NK and CTL activity and IFN-γ expression during the primary immune response to ECTV-IL4(TK+) and the reported inhibitory effect of IL-4 on the Th1-mediated DTH response (34), we next investigated the effects of ECTV IL-4 expression on the memory response to ECTV in immune mice. Immunization of both C57BL/6 and BALB/c mice with the attenuated TK^- virus ECTV-602 caused a mild swelling at the inoculation site which resolved within 14 day p.i., with all mice recovering from infection (16). Twenty-eight days postimmunization, the mice were challenged with either control ECTV-602(TK+) or ECTV-IL4(TK+). These immune mice displayed greatly differing DTH responses to infection (Table 2). Mice challenged with the control virus displayed a mild DTH response at 24 h, which was resolving by 48 h postchallenge, and viral titers in the inoculated footpads were below the detection limits of the viral plaque assay 7 days p.i., indicating that these mice were immune to infection with ECTV. Immunized mice were also immune to reinfection with the virulent Moscow strain (data not shown). In contrast, mice challenged with ECTV-IL4(TK+) displayed an exacerbated response characterized by extreme swelling of the inoculated foot 24 and 48 h p.i. (Table 2). The difference in footpad swelling between C57BL/6 and BALB/c mice 24 h after challenge with ECTV-IL4(TK+) is significant by Student's *t* test ($P < 0.01$); however, there was no significant difference in footpad swelling between these strains 48 h postchallenge. Twenty-four hours postchallenge, hematoxylin-and-eosin-stained footpad sections of ECTV-IL4(TK+)-infected mice showed an enhanced lymphocyte infiltrate in the inoculated foot relative to the DTH response of the control mice (data not shown). The inoculated feet continued to swell, and 60% of the mice died between days 6 to 8 p.i. At death, the livers and spleens of these mice were enlarged and contained numerous discrete local lesions, suggestive of death due to acute mousepox. Animals which survived challenge with ECTV-IL4(TK+) were autopsied on day 21 p.i., at which time they still displayed marked swelling of the inoculated feet. Viral titration of tissues isolated from surviving mice indicated they were controlling systemic infection, since virus was not detected in the spleen. However, virus clearance was considerably delayed at the site of inoculation. Average titers of virus in the inoculated feet of the surviving C57BL/6 and BALB/c mice 21 days p.i. were 6.0×10^3 and 1.3×10^5 PFU/g of tissue, respectively.

DISCUSSION

Footpad inoculation of naive mice with a recombinant TK^+ ECTV expressing mouse IL-4 results in systemic infection and suppression of splenic NK and CTL cytolytic activity and IFN-γ expression. The expression of IL-4 by ECTV renders the virus lethal to mice that are normally genetically resistant. Importantly, memory responses in mice previously immunized with ECTV were also inhibited, leading to uncontrolled viral replication in the visceral organs and resulting in classic symptoms of acute mousepox.

Previous studies using the closely related orthopoxvirus VACV have established that IL-4 coexpression is associated with a delay in virus clearance from the major target organs (2, 41). This is likely to be the result of the observed combined reduction in antiviral effectors such as $CD8^+$ CTL precursor (CTLp) development, IFN-γ expression, macrophage activation, and inducible nitric oxide production (4, 41). While no reduction in splenic NK cell activity was observed in infected CBA mice (41), a significant reduction was found when SCID mice were infected with VACV expressing IL-4 (4). It is clear from the VACV model that IL-4 down-regulates in vivo expression of the key type 1 cytokines IL-2, IL-12, and IFN-γ (41), which are pivotal for stimulation and differentiation of antiviral cell-mediated effectors such as NK, $CD8^+$ CTL, and $CD4^+$ Th1 cells (3, 40).

In the classical ECTV pathogenesis study conducted by Fenner (12), replicating wild-type Moscow strain of ECTV can first be detected in the liver and spleens of mice by 3 days p.i. At this stage in the present study, virus-encoded IL-4 had a significant effect on the cytolytic activity of NK cells in these organs. The observed partial down-regulation of NK cell activity seen here is likely to be the result of the combined IL-4-induced suppression of IL-12 expression by antigen-presenting cells (7, 8, 25) and type 1 cytokine receptors on NK cells (30, 33, 39, 45) inhibiting activation and proliferation. In the VACV IL-4 infection model, IL-12 expression in the spleen was clearly down-regulated by day 2 p.i. (41). The remaining NK cell cytolytic activity observed during infection with ECTV-IL4(TK+) probably results from the more usual mode of IFN-α/β induction generally seen during viral infections (3).

Later, at day 7 p.i., when the adaptive anti-ECTV cell-mediated response in the spleen should be near maximal (29), there was no measurable $CD8^+$ cytolytic activity or IFN-γ expression by isolated splenocytes of mice infected with ECTV-IL4(TK+). Viral replication appeared to be uncontrolled, with the mice dying shortly thereafter. This is consistent with the IL-4-induced suppression of IL-12/IFN-γ expression inhibiting CTLp development (41). Under some circumstances, stimulation of naive $CD8^+$ cells in the presence of IL-4 can generate noncytotoxic Tc2 cells (10, 11). In addition, in vitro IL-4 treatment of activated Tc1 cells has been shown to cause defective cytokine expression and inhibit proliferation in response to antigen stimulation (37). Abnormally high IL-4 levels, accompanied by suppressed production of IL-12 and IFN-γ, could potentially result in the generation of either Tc2 cells with reduced cytolytic activity or defective Tc1 cells, which may also account for the observed reduced $CD8^+$ lytic activity and IFN-γ expression following infection with ECTV-IL4 (TK+). In marked contrast to the VACV IL-4 model, however, infection with ECTV-IL4(TK+) and suppression of $CD8^+$ effector function appears to be absolute, which could be a consequence of the greater ability of ECTV to replicate in the mouse, with higher and sustained levels of IL-4 expression compared to VACV.

It is also possible that high levels of ECTV-IL4(TK+) replication in the spleen may have resulted in enhanced lymphocyte killing, accounting for the observed reduction in cytotoxic activity and IFN-γ expression. However, IL-4 expression by poxviruses is not known to confer increased replicative ability in vivo. Indeed, peak titers of VACV-IL4 in the ovary (41) and spleen (2) or of attenuated ECTV-IL4 at the inoculation site (C. D. Christensen, R. J. Jackson, and A. J. Ramsay, unpublished results) are equivalent to those seen in infection with control virus.

A similarly constructed TK^- ECTV expressing IL-4 was attenuated upon infection of the naive CBA mice, and replication of this virus was restricted to the inoculated footpad, accompanied by extreme swelling (Christensen et al., unpublished). More importantly, there was no measurable reduction in the generation of splenic cytolytic lymphocyte or IFN-γ responses compared to control virus infection, although clearance of the virus from the inoculated foot was again considerably delayed. This suggests that the induced suppression of the antiviral NK, CTL, and IFN-γ responses observed in the present study was localized to the site of viral expressed IL-4 in the lymphoid tissue. It has previously been shown that the TK^+ phenotype of ECTV is required for replication in macrophages, allowing dissemination from the site of inoculation and viral replication in the liver and spleen (26). In the present study, dissemination of ECTV-IL4(TK+) to the visceral organs, followed by systemic expression of IL-4, suppressed development of cell-mediated cytotoxic responses in the lymphoid tissues, culminating in uncontrolled viral replication, acute organ failure, and death.

It is clear from these studies that IL-4 expression also permits uncontrolled viral replication in the visceral organs of ECTV-immune mice, indicating that this factor can inhibit the generation of effector cells from a pool of memory T cells. In contrast, preexisting immunity to ECTV was sufficient to limit reinfection with either control or virulent Moscow virus. T-cell immunological memory is considered to result from enhanced numbers of antigen-specific CTLp and residual populations of activated CTL effector cells (9). Thus, even in the presence of preexisting immunity, IL-4 can inhibit the expression of immunological memory. These findings demonstrate the effectiveness of IL-4 for the inhibition of powerful cell-mediated immune reactions and suggest strategies potentially useful for the control of deleterious immune responses, such as autoimmune reactions.

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