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The 'supervirus'? Lessons from IL-4-expressing poxviruses

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Members of the *Poxviridae* family are particularly adept at avoiding the host immune system, encoding a plethora of immunomodulatory proteins that subvert host defense. With their large genome, poxviruses are also useful for studying the effect of exogenous genes on virus–host interactions and immune responses. The insertion of the Th2 cytokine interleukin-4 (IL-4) into several poxviruses significantly increases the efficiency of the recombinant virus as a pathogen by directly inhibiting the development of Th1 immunity, which is crucial for viral clearance. In an age in which the fear of genetically modified weaponized pathogens exists, the understanding of how to make viruses more pathogenic further blurs the distinction between fundamental academic research and bioweapons development. Here, the extent of immune evasion by IL-4-expressing poxviruses will be explored, as will the consequences of this increased pathogenicity on protective immune responses.

A fine balance: Th1/Th2 immunity and poxviruses

The dichotomy between immune responses that are predominated by $CD4^+$ T cells of the Th1 and Th2 subtypes has been well established and extensively studied (reviewed in Refs [1–4]). A cellular response predominated by Th1 cells generally results in a polarization towards cell-mediated [cytotoxic T-lymphocyte (CTL)] effector functions, whereas those predominated by Th2 cells are skewed more towards antibody-mediated reactions (Box 1). The resultant immune responses are profoundly influenced by the production of characteristic subsets of cytokines, which are produced by these T cells. There is also a degree of mutual exclusion between these two subsets, under the influence of key cytokines that are generated by one branch, and which inhibit the generation of Th cells of the opposing type [2,5]. In addition, resistance or susceptibility to pathogens is strongly determined by the balance of Th1 and Th2 cytokines produced during infection [6–8]. In general, a successful antiviral immune response is characterized by the elimination of virus-infected cells through the actions of $CD8^+$ CTLs, which are generally generated with help from Th1 cells.

The poxviruses are a large family of double-stranded DNA viruses that are notable for their ability to replicate in the cytoplasm of infected cells autonomously of the host nuclear machinery and the propensity to express a wide spectrum of immunomodulatory proteins [9,10] (Table 1). The antiviral host CTL response to poxviruses is dependent on the production of interferon-γ (IFN-γ) and other Th1 cytokines, which contribute as effector molecules in the successful clearance of the infection [11,12]. The early production of Th2 cytokines, and interleukin-4 (IL-4) in particular, inhibits the development of CTLs from precursor cells and can even promote the development of $CD8^+$ T cells that lose their cytotoxic capacity [13]. IL-4 has been ectopically expressed from recombinant viruses, such as retroviruses [14], herpes simplex virus [15,16] and vesicular stomatitis virus [17], with little effect on virus infectivity and pathogenesis within its host. However, for poxviruses, the expression of IL-4 by a recombinant virus can significantly inhibit the ability of the host to clear this pathogen, tipping the balance in favor of the virus (Table 2). Here, we consider the implications of modifying the host immune responses to poxviruses expressing the immunomodulatory cytokine IL-4.

Box 1. The Th 1/Th 2 paradigm

The finding that $CD4^+$ T cells could be functionally divided into at least two distinct subsets was important in the understanding of how the immune response could be functionally directed against different pathogens. Initially, it was discovered that MHC II-restricted T cells could be functionally classified into two groups, designated Th1 and Th2, based on their production of characteristic cytokines. Th1 cells produce IFN-γ, TNF-α and IL-2 and Th2 cells produce IL-4, IL-5, IL-10 and IL-13 [55]. Th1 cells are predominantly involved in priming the responses of $CD8^+$ T cells and dominate immune responses against viruses and intracellular bacteria. Th2 cells are involved in B-cell priming and are prominent in the control of parasitic infections. There is a reciprocal negative regulation of the development of these subsets, modulated by the cytokines produced. The original Th1/Th2 paradigm has been adjusted since its discovery nearly two decades ago [55]. MHC I-restricted cells can also produce characteristic patterns of cytokines (designated Tc1 and Tc2) and cells of the dendritic, monocyte/macrophage and NK-cell lineage, which can be similarly separated. There are also an additional subset of T cells designated regulatory T cells, a heterogeneous population of T cells that are capable of exerting negative control on both Th1 and Th2 cells. In addition, newly studied cell types, such as NKT cells, appear to be an important component of immune system regulation. Therefore, what was considered to be a fairly straightforward paradigm, with Th cells acting as central regulators, has developed into a complex system of regulation and cross-regulation by a variety of cell types.

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Table 1. Selected immunomodulatory genes of several representative poxviruses[a]

Immunomodulator	Ectromelia (ECTV)[b]	Myxoma (MYXV)[c]	Vaccinia (VACV)[d]	Variola (VARV)[e,f]
IFN-α/β inhibitor	*ORF 166*	*M135R*	*B19R*	*B20R*
IFN-γ inhibitor	*ORF 158*	*M007R/L*	*B8R*	*B9R*
eIF2α mimic	–	*M156R*	*K3L*	*C3L*
dsRNA-BP[g]	*ORF 043*	*M029L*	*E3L*	*E3L*
TNF inhibitor	–	*M002R/L*	*B28R*	*G2R*
TLR inhibitor	*ORF 145*	–	*A46R, A52R*	*A52R*
IL-18 BP	*ORF 013*	–	–	*D5L*
Chemokine BP	*ORF 001/172*	*M001R/L M007R/L*	*B29R*	*G3R, A46L*
GM-CSF or IL-2 inhibitor	–	–	–	*A46R*
Serpins	*ORF 168, ORF 161*	*M152R, M151R, M008.1L*	*C12L, B13R/B14R, K2L*	*B13R, B25R, C2L*

[a]Table adapted from Refs [9,53].
[b]Strain Moscow, gene designations from Ref. [29].
[c]Strain Lausanne, gene designation represents myxoma (M) and ORF designation.
[d]Strain Copenhagen, gene letter designations represent restriction digest fragments.
[e]Strain India, 1967, gene letter designations represent restriction digest fragments.
[f]Untested Variola immunomodulators are presumed to share some properties of orthologous versions from other poxviruses.
[g]Abbreviations: BP, binding protein; eIF2α, eukaryotic translation initiation factor 2 subunit α; GM-CSF, granulocyte–macrophage-colony stimulating factor; TLR, Toll-like receptor.

Vaccinia virus expressing IL-4

The role of Th1 versus Th2 immune responses in poxvirus infection has been extensively studied in the mouse using vaccinia virus (VACV), the orthopoxvirus vaccine used in the eradication of variola major (VARV) (causative agent of smallpox). Currently, the natural host of VACV is unknown, yet this virus can productively infect many vertebrate species, ranging from mice to humans [11,18]. Inbred mice can contain and clear VACV infection, with no particular genetic susceptibility in fully immunocompetent hosts [5]. Using mice genetically deficient in cytokines, researchers have examined the role of different regulatory cytokines *in vivo* following VACV infection. Mice deficient in IL-2, IL-12, IFN-γ, IFN-γR, IFN-αβR, tumor necrosis factor receptor (TNFR) and IL-6 are all generally more susceptible to infection with VACV than wildtype mice, whereas a deficiency in Th2 cytokines, such as IL-4 and IL-5, has little effect [19].

In contrast to studies in mice lacking specific cytokines, research has also been focused on the introduction of cytokines into VACV, examining the effect of their expression concurrent with primary virus infection. This approach has exploited the large genome of poxviruses and the ability to insert foreign genes through homologous recombination [20]. Used extensively in gene therapy, with a well documented history of use in humans, VACV is an ideal candidate to study the ectopic expression of cytokines for therapeutic purposes [21,22].

Table 2. Poxviruses expressing IL-4

	VACV-mIL-4	ECTV-mIL-4	MYXV-rIL-4
Host	Mouse	Mouse	Rabbit
Delay in viral clearance	Yes	Yes	Yes
Causes disease in resistant hosts	N/A[b]	Yes	Yes
Causes disease in vaccinated hosts	No	Yes	No
Immunizing virus	MVA	ECTV-602(TK-)	Ur-HA
Targeted immune response	CTLs		
Mononuclear phagocytes	CTLs, NK cells	CTLs, NK cells[a]	
Refs	[23,24,40,54]	[32]	[44]

[a]Effect inferred based on mouse studies.
[b]N/A, not applicable (i.e. virus does not have a 'natural host').

In mice, the expression of murine IL-4 from recombinant VACV results in a decrease in CTL levels and a delay in viral clearance [23,24]. The decrease in CTL response is caused by a 12-fold reduction in CTL precursor (CTLp) frequency [19]. The increase in virulence was also ascribed to an inhibition of IL-12, IL-2, IFN-γ and nitric oxide (NO) production, yet the expression of IL-4 has little effect on antibody or natural killer (NK)-cell levels [24].

The effect of IL-4 on CTL levels has also been demonstrated by creating recombinant VACV that express various antigens in the presence of IL-4. When IL-4 is expressed in VACV in combination with the respiratory syncytial virus (RSV) F protein, there is a delayed clearance of the IL-4-expressing virus compared to the virus not expressing this cytokine [25]. Expression of IL-4 generates RSV-specific antibodies skewed to the IgG1 subtype, yet does not inhibit the development of RSV-specific memory CTLs or IFN-γ production by RSV-re-stimulated spleen cells. Another related study examining the M2 epitope of RSV reported that mice immunized with VACV, expressing this epitope in combination with IL-4, have a lower M2-specific CTL response accompanied by lower intracellular IFN-γ production and diminished $CD8^+$ RSV-specific responses *in vivo* [26]. Subsequent experiments have shown that infection of mice with VACV expressing M2 plus IL-4 results in increased FasL expression on T cells of infected mice and causes a shift in the mechanism of CTL killing from an exocytosis (perforin-mediated) to a Fas–FasL-mediated killing pathway [27].

One elegant study examined the effect of IL-4 expression by VACV on the CTL response, using a TCR transgenic system [28]. Transgenic H-$2K^b$ mice specific for ovalbumin (OVA) peptide were immunized with either VACV–OVA or VACV–OVA–IL-4 [19,28]. IL-4 expression reduces the OVA peptide-specific CTL response significantly and is associated with a decrease in the proliferation of OVA-specific CTLs. However, on a per cell basis,

the production of IFN-γ and the cytotoxicity of OVA-specific CTL cells are not affected [28]. This would indicate that the reduction in CTL responses caused by IL-4 is due to the reduced numbers of antigen-specific effector cells as opposed to the downregulation of the effector functions of these cells.

Ectromelia expressing IL-4: 'super mousepox'?

Ectromelia virus (ECTV) is an orthopoxvirus that is a close relative of VARV and VACV and is a natural pathogen to mice, causing mousepox [29]. ECTV virulence is dependent on the host genetic background and is influenced by several parameters, with at least four genetic loci being involved (reviewed in Ref. [5]). The highly susceptible mouse strains (including Balb/c, A/J and DBA/2) exhibit 100% mortality to ECTV infection, whereas in genetically resistant strains (including C57/BL6 and certain 129 strains), virus infection causes a rapid induction of the Th1 cytokines IL-2, IFN-γ and TNF-α, resulting in virus clearance [5]. The recovery of genetically resistant mouse strains from primary ECTV infection is dependant on the combined actions of mononuclear phagocytes, NK cells and CTLs [30]. It has recently been suggested that an antibody-mediated response is important for later stages of recovery from virus infection [31], yet there is little difference in the induction of Th2 cytokines, such as IL-4, in either genetically susceptible or resistant strains. In addition, as was found using VACV, mice genetically deficient in IL-4 demonstrate no difference from wildtype controls in their ability to mount a specific antiviral CTL response or to clear VACV infection. ECTV has the advantage over VACV in that it is a natural pathogen with clearly defined genetic susceptibility in mice, therefore it enables a more detailed study of the effect of exogenous IL-4 in the mouse model.

A pivotal report, examining the expression of IL-4 during the course of a natural poxvirus infection, demonstrated an important role for this cytokine in dampening the mouse immune response to ECTV, creating an ECTV with exacerbated pathogenicity [32]. The original focus of the research in engineering ECTV with IL-4 was to develop immunocontraception for the mouse population in Australia [33]. For this purpose, Jackson and colleagues [32] generated a recombinant ECTV expressing the mouse eggshell protein zona pellucida 3 (ZP3). A strong antibody response to this protein should result in the destruction of fertilized eggs in female mice but the ECTV–ZP3 construct was so ineffective in C57/BL6 mice that the group examined the consequences of enhancing the Th2 response using IL-4 [32]. A similar approach, without the use of IL-4, had previously been moderately successful as immunocontraception in Th2-biased Balb/c mice [34]. The expression of murine IL-4 by recombinant ECTV suppressed the cytotoxic lymphocyte responses and overcame the natural genetic resistance of the C57/BL6 mouse to ECTV, causing a disease in these mice that is strikingly similar to that caused by wildtype ECTV in susceptible mouse strains [32]. The cytolytic activity of splenocytes from mice infected with ECTV–mIL-4 was severely defective in their ability to lyse infected targets and a threefold decrease in NK-cell activity was noted [32]. Most remarkably, however, re-challenge of recently immunized C57/BL6 mice (immunized with the attenuated ECTV–602 TK^- strain) with ECTV–mIL-4 also results in significant mortality (three out of five mice) due to fulminate mousepox [32], whereas all mice challenged with ECTV–602 (TK^+) or the more virulent Moscow strain of ECTV are protected by immunization (Table 2). At face value, this would indicate that this IL-4-expressing virus could inhibit the effector arm of immune memory responses, a finding that was not observed with VACV that expresses IL-4. Recent studies have examined the potential treatment of these more virulent ECTV–IL-4 infections in mice using antiviral therapies [35] (R.M.L. Buller, personal communication).

Why the difference between IL-4-expressing VACV and ECTV in mice?

The extreme nature of the immunosuppression in mice that is caused by infection with ECTV expressing IL-4 is somewhat surprising. Previous studies using VACV expressing IL-4 had shown that IL-4 co-expression is associated with a delay in the virus clearance from major organs, probably due to the combined reduction of antiviral effectors, such as IFN-γ and inducible nitric oxide synthase (iNOS), and is linked to a decrease in CTLp and activated macrophages [24,36]. Thus, it is likely that ectopic IL-4 expression acts to increase the pathogenesis of poxviruses by a combination of T cell-dependent and T cell-independent effects.

A recent paper [31] examined the relative Th1 versus Th2 immune responses, in both resistant and susceptible mouse strains, to ECTV infection. In resistant strains infected with ECTV, such as C57/BL6, there is a strong Th1 response (associated with robust production of IFN-γ, IL-2 and TNF) within the first few days after infection that is linked to a strong induction of antiviral CTLs, which facilitate viral clearance. Within susceptible strains infected with ECTV, there is generation of a Th2 response (with IL-4 production and low IL-2 and IFN-γ production) that results in weak CTL responses and uncontrolled virus replication and death [31]. However, both the resistant and the susceptible mice have similar numbers of IL-4-producing cells after infection, indicating that the lack of IFN-γ production appears to be a deciding factor in the increased susceptibility to ECTV seen in Balb/c [31].

It has been suggested by Jackson and others that the highly lethal consequences of expressing IL-4 is peculiar to ECTV and is linked to its production of a soluble IFN-γ-binding protein [32,33,37]. Studies in mice have shown that when IFN-γ is neutralized by antibody, there is a dramatic increase in the viral load and a delay in ECTV clearance [19]. In resistant C57/BL6 mice, the depletion of IFN-γ results in a lethal infection [38]. This would argue against the idea that ECTV encodes an IFN-γ binding protein that fully accounts for its increased virulence. In addition, studies that have examined mice genetically deficient in the perforin-mediated CTL pathway report a significant difference in their ability to clear VACV and ECTV in the absence of IFN-γ [19]. Using an adoptive transfer system, with immune cells generated from mice

that are deficient in either perforin or IFN-γ, IFN-$\gamma^{-/-}$ mice were immunized with a virulent ECTV or VACV [19]. In response to ECTV, IFN-γ-deficient cells clear the virus as effectively as wildtype effectors, yet perforin-deficient effectors cannot. However, the control of VACV appears to occur independently of perforin production and is crucially dependent on IFN-γ [19]. These results indicate a fundamental difference in the ability of the mouse immune system to recognize and control these two different poxviruses and suggests that there is a crucial step in which the ectopic IL-4 can tip the balance towards virus dominance.

These experiments also indicate that it is the CTL-mediated killing pathway, and not the neutralization of IFN-γ, that is crucially important in the clearance of ECTV. IL-4 might cause a switch in the cytolytic mechanism of cytotoxic T (Tc) cells from an exocytosis (perforin)-mediated pathway to a pathway mediated by the Fas–FasL-mediated pathway of targeted cell death [27,39], which is supported by the results discussed here. This is combined with the finding that many orthopoxviruses, including ECTV, express intracellular serpins that target the Fas–FasL pathway [9]. There is also evidence that $CD8^+$ T cells stimulated in the presence of IL-4 can develop into CTLs with decreased cytotoxic activity [13]. All of this suggests that an excess of IL-4 could severely impair the ability of the host immune system to mount a successful antiviral CTL response.

In addition, other studies suggest that IL-4 expression can exacerbate disease in a manner that is independent of T cells. For example, a group examining VACV as a method of gene delivery found that expression of IL-4 by VACV exacerbates disease through a decrease in macrophage cytotoxic activity, potentially through the suppression of IFN-γ production by NK cells [36]. Thus, the inhibition of the immune response following VACV infection might be more IFN-γ oriented, whereas the response to ECTV might be more dependent on an appropriate CTL response, accounting for some of the differences between these two viruses seen in mouse models.

In contrast to the immunization studies with ECTV [32], recent reports have indicated that immunization of mice with the attenuated VACV, modified vaccinia virus Ankara (MVA), can provide effective immune protection against the replication-competent VACV vSC8, as well as vSC8-IL-4 [40]. This protection is seen even in the presence of T- and B-cell depletion, indicating that the protection provided by MVA immunization is both redundant and complex [40]. The difference between the protective immune response to VACV and ECTV might be significantly different in mice, thus it is plausible that ECTV expressing IL-4 could inhibit the effector arm of memory immune responses induced by vaccination with ECTV but not VACV. This inhibition is probably owing to the creation of a microenvironment in which productive memory CTL responses are unable to exert their antiviral effects, which is an area requiring further study.

Myxoma virus expressing IL-4

The effect of ectopic IL-4 expressed by a leporipoxvirus has also been examined using myxoma virus (MYXV) and the European rabbit (*Oryctolagus cuniculus*) (Table 2). The native hosts of MYXV are rabbits of the Sylvilagus genus, in which the virus causes a benign disease, yet in the European rabbit, MYXV causes lethal myxomatosis [41]. However, since the introduction of MYXV into the Australian wild rabbit population as a means of biological control in 1950, there has been an ongoing co-evolution of both the virus and host, resulting in a less virulent virus, which is more effectively transmitted by mosquito vectors, and a genetically resistant rabbit population [42,43]. This natural genetic resistance of a host species provides a unique model in which to study the consequences of expressing IL-4 from the MYXV genome.

The *IL-4* gene was inserted into the virulent SLS (standard laboratory strain) of MYXV (the original release strain), as well as the more attenuated Uriarra-2–53/1 strain (Ur) that was isolated in Australia in 1953, two years after the release of SLS. The virulence of these viruses was examined in the susceptible laboratory strain of rabbits (*Oryctolagus cuniculus*), as well as genetically resistant wild rabbits. Kerr *et al.* observed that insertion of IL-4 into the virulent SLS strain overcame the genetic resistance in the wild rabbits and resulted in fatal myxomatosis [44]. Insertion of IL-4 into the Ur strain also resulted in fatal myxomatosis in the susceptible laboratory rabbits, although it was no more virulent in the resistant wild rabbits than the SLS. The authors speculate that the increase in virulence of MYXV–rIL-4 is a result of similar effects on the rabbit immune system as the ECTV expressing mIL-4 in the mouse. One significant difference between these two studies is that the insertion of IL-4 into MYXV does not affect immunity induced by earlier vaccination [44]. It will be important to determine the differences in the host immune responses to ECTV and MYXV because these will give many important clues as to the effect of ectopic IL-4 expression on both the virulence of the virus and its direct effect on the immune response and resulting pathology.

Potential for 'super-smallpox'?

The fear in the modern age of biowarfare is that viruses could be genetically modified to become more virulent or even break pre-existing immunity. Because the immune response parameters that result in the successful clearance of VARV in VARV-vaccinated humans is largely unknown, researchers depend heavily on animal models and selected pathogenic poxviral infections to infer how the causative agent of smallpox might be manipulated and the resultant effects on human hosts. In addition, the global immunity to smallpox has declined significantly in the past 20 years and there is a noticeable lack of significant immunity in most people born after 1970. The current vaccines for smallpox, such as Dryvax©, are contraindicated for a growing number of people, including those who are immunocompromised and those with skin conditions, such as atopic dermatitis (AD), psoriasis and eczema. Interestingly, the contraindication of live vaccinia virus in AD patients appears to be directly linked to strong Th2 immune response in the skin [45]. However, second-generation vaccines, such as MVA, have shown promise, being protective against pulmonary VARV challenge in

animal models [46] as well as successfully protecting mice against VACV expressing IL-4 [40].

The pivotal question remains: could ectopic IL-4 expressed from a recombinant VARV be as detrimental in human hosts, as in some of the animal models? By all indications, the expression of IL-4 in the context of a poxviral infection, in which the virus itself encodes a variety of its own repertoire of gene products that significantly inhibit the host immune response [9], should be a selective advantage for the virus (Table 1 and Figure 1).

Yet, many key questions remain unanswered (Box 2). For one, it is difficult to predict how experiments on inbred mouse models will translate to the immune response of outbred human populations [47,48]. Experiments using VACV to express caprine (goat) IL-4 show no difference in the overall immune response of outbred animals *in vivo* [49]. In addition, the host response to ECTV in the mouse appears to be dependant on the generation of $CD8^+$ CTLs, and at this point, the relative importance of the cell-mediated versus the humoral response in VARV infection in humans is unknown. Recently, a new model to study the secondary viremia phase of VARV infection in macaques was reported [50]. Infected peripheral blood mononuclear cells from these animals exhibit upregulation of a high proportion of Ig genes, as assessed by gene microarray, indicating that a strong antibody response could be important sequelae to VARV infection [51]. However, this model might more closely mirror the secondary viremia stage of the universally fatal hemorrhagic form of smallpox than the more classical disease. But it is difficult to predict the possible effects of enhancing Th2 immunity in the context of this complex pathogen. Experiments in more complex models, such as the macaque, should help elucidate what a successful immune response directed against pathogenic orthopoxvirus infection actually involves. In addition, it should give insight into how manipulating the formation of the immune response within the context of poxvirus infection can help tip the balance in the favor of either host or pathogen. It is also crucially important to understand how vaccination is affected by genetic manipulation of poxviruses because our pre-existing immunity stands to be our last line of defense.

Is the Th1/Th2 balance sufficient to explain the increased virulence of IL-4-expressing poxviruses?

It is clear from studies in a variety of IL-4-expressing poxviruses that the ectopic expression of this cytokine in the context of virus infection inhibits the formation of antiviral CTL responses. However, is the effect on T cells sufficient to explain the exacerbated lethality of these viruses? The effect of IL-4 expressed by ECTV on pre-existing immunity would suggest that expression of this cytokine modifies the microenvironment, such that protective immune responses cannot properly function [32].

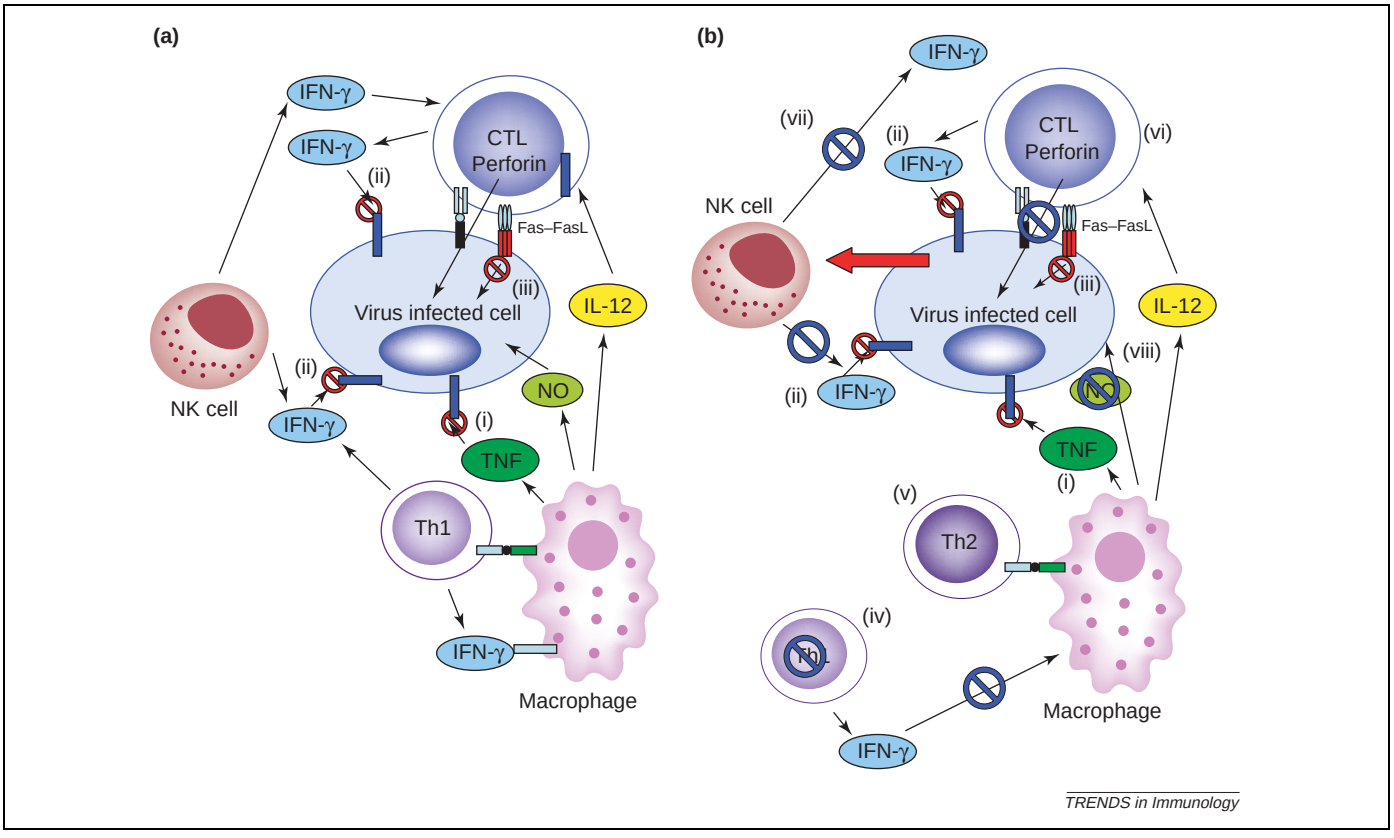

Figure 1. The effect of poxvirus IL-4 production on the host immune response. **(a)** The immunology of poxvirus infection is complex. Poxvirus infection is a strong inducer of antiviral proteins, such as IFNs, NO and TNF. However, these viruses also encode many proteins that modulate the host immune response (indicated as red circles with a line through them), including (i) TNF, (ii) IFN receptor analogues and also serpins (iii), which inhibit the killing actions of death effectors, such as Fas–FasL. **(b)** The introduction of IL-4 (red arrow) into a poxvirus adds another layer of complexity onto the host immune modulation (blue circles with lines through them). Exogenous IL-4 (iv) inhibits the development of Th1 cells, (v) resulting in a Th2-dominated immune response and (vi) inhibiting the development of virus-specific CTL effector responses. In addition, there is (vii) suppression of IFN-γ production by NK cells and (viii) a decrease in the cytotoxic activity of macrophages.

Box 2. Outstanding questions

1. What is the effect of promoter selection on the relative virulence of IL-4-expressing poxviruses?
2. Does the most efficacious human immune response against poxviruses involve predominant CTL- or antibody-mediated effectors?
3. What other effects does IL-4 expression have on other components of the immune system, such as cells of the monocyte/macrophage lineage?
4. Is ectopic IL-4 expression contributing to an immunopathology separate from inhibiting antiviral CTL responses?
5. Does IL-4 expression from recombinant poxviruses contribute to a 'cytokine storm' from hyper-activated myeloid cells?
6. Should an IL-4-expressing monkeypox recombinant be created to address whether IL-4 expression can alter either pathogenicity or the ability to circumvent vaccination in primates?

In addition, the appearance of an exaggerated disease in rabbits immunized with MYXV–IL-4 that includes clinical symptoms not associated with myxomatosis, such as muscle trembling, respiratory difficulty and coma, and the appearance of gastric ulcers, suggest that the IL-4 might contribute to an immunopathology that is partially independent on its effect on the antiviral response [44]. Finally, the finding that expressing antigens, such as influenza HA, in the context of VACV that expresses IL-4 fails to enhance the antibody response to either VACV or the antigen HA would suggest that ectopic IL-4 expressed in this context is not acting solely as a classical Th2-promoting cytokine [23].

We propose that there needs to be a closer examination of other immune cells whose function could be drastically altered by IL-4. These include cells of the monocyte/macrophage lineage, which are intimately involved in the generation of antiviral responses through the production of cytokines and the presentation of antigen as well as the production of antiviral effectors, such as NO. Myeloid lineage cells have also been recently shown as the primary site of VARV replication in infected macaques [50]. Macrophage activation and polarization are also affected by the cytokines in the microenvironment (reviewed in Ref. [52]) and it will be important to see how modulation of macrophage function through the expression of IL-4 within the infected tissue microenvironment might influence the host antiviral immune response. It is possible that the balance struck by the immune system in poxvirus infection is indeed a tenuous one, and cytokine interference might have sweeping effects that could result in an exaggerated immune response and subsequent immunopathology. Thus, the effect of ectopic IL-4 expression by poxviruses might include more global effects on the immunopathology, as well as more localized effects on immune cells that bridge the gap between the innate and adaptive immune system, such as the tissue macrophage. This issue merits further attention because the most robust defense against the possible exploitation of recombinant viruses for malicious purposes is further research into the basic mechanisms of how viral pathology can be manipulated, and, hopefully, interdicted.

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