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***J. Virol.* 2001, 75(18):8353. DOI: 10.1128/JVI.75.18.8353-8355.2001.**

JOURNAL OF VIROLOGY, Sept. 2001, p. 8353–8355 Vol. 75, No. 18
0022-538X/01/$04.00+0 DOI: 10.1128/JVI.75.18.8353–8355.2001

MINIREVIEW

Creation of Killer Poxvirus Could Have Been Predicted

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Genetic engineering of viruses has become a common practice. One major objective is to use engineered viral vectors for the delivery of genetic information with therapeutic intent or to modify viruses so as to alter the host's immune response to it. Changes in virulence and host range of modified viruses are generally hard to predict. One such virus was recently described in an article published in the *Journal of Virology* (8) which received wide coverage in both the scientific (7, 8, 15) and popular press, as well as the electronic media.

The reason for the heightened public interest in this publication was the implication that a means had inadvertently been discovered to allow the creation of killer viruses with potential biological warfare application. According to some of the authors of the above study, "this came as a complete surprise and was totally unexpected." However, a closer reading of the literature on the consequences of infection with recombinant poxviruses encoding interleukin-4 (IL-4), some performed by members of the above study or former colleagues, suggests otherwise.

Ectromelia virus (Ect), an orthopoxvirus and close relative of variola virus (smallpox) and vaccinia virus (VV) (the poxvirus used to vaccinate against smallpox), is a natural mouse pathogen and causes mousepox. Virulence depends on the host genetic background and ranges from the highly susceptible A/J strain of mice (50% lethal dose [LD_{50}] of 0.04 PFU) to the relatively resistant C57BL/6 mice (LD_{50} of $>10^5$ PFU) (5). Recovery from Ect infection in the genetically resistant strain is absolutely dependent on the cytotoxic T (Tc) cell-mediated exocytosis pathway of cytolysis and in particular on the presence of granzymes A and B (10, 12). VV, on the other hand, is much less virulent, and only causes mortality in mice and humans in immunocompromized individuals or by using very high doses ($>10^7$ PFU) in mice.

VV has become one of the most frequently used vectors for the expression of genes from pathogens and mammalian genes in mammalian cells in vivo and in vitro. To improve or alter the host's immune response to VV itself and or to the products of foreign inserted genes, additional genes coding for cytokines have been added to the VV genome (14). The rationale behind including IL-4 in such constructs was the assumption that a type 2 cytokine such as IL-4 would skew the immune response to an elevated antibody response at the expense of a Tc cell response. Early work by Andrew and colleagues (1, 2) reported a diminished Tc cell response to VV and recombinant influenza virus hemagglutinin (HA) after immunization with VV recombinants encoding IL-4. In addition and contrary to expectations, no enhancement in the antibody response to either VV or antigen HA was observed. Most important is their comment: "however, this paper demonstrates that IL-4 is unlikely to increase vaccine efficacy and for the first time documents that IL-4 can be lethal when administered in vivo" (1).

Results verifying these initial studies of decreased lytic activity in splenocytes after immunization with VV-IL-4 recombinants have been documented (4, 15). A role for IL-4 in downregulation of cytolytic activity of splenocytes is supported by experiments using mice lacking IL-4 expression (20) or transgenic mice overexpressing IL-4 (7). The generally accepted interpretation of the mechanism responsible for the reduced observed Tc cell activity was that IL-4 caused immune deviation from a type 1 response to a type 2 response (6). However, a very recent study by Aung and Graham (3) suggests that a different mechanism other than immune-class deviation may be involved or at least contribute to the observed diminished Tc cell activity when IL-4 recombinant viruses are used as immunogens. They showed convincingly that rather than diminished Tc cell responses after immunization with VV-IL-4 recombinants, the antigen-specific lysis of target cells was dependent on the expression of Fas on target cells. Thus, it appears that the presence of IL-4 switches the cytolytic mechanism of Tc cells from the exocytosis (perforin and granzyme)-mediated pathway to the Fas/Fas ligand-mediated pathway of target cell death. Thus, cytolytic effector function should theoretically be operative, although it may be delayed. This assumption is based on recent published studies showing that target cells not expressing Fas do become Fas positive in the presence of Tc cells. This mechanism was identified by using Tc cells from perforin-deficient mice (17).

The observed failure of poxvirus-preimmunized mice to be protected from a subsequent challenge with the Ect-IL-4 recombinant virus (8) strongly suggests that memory Tc cells are vital for protection from secondary poxvirus infections. In addition, it indicates that activation of memory Tc cell precursors by antigen-presenting cells expressing IL-4 is modulated similarly, as are naive Tc cell precursors.

The caveat in the case of orthopoxvirus infection is that a number of orthopoxviruses have been shown to inhibit Tc cell-mediated killing of target cells, predominantly via the Fas pathway (9, 11, 18). This inhibition is mediated by a poxvirus-

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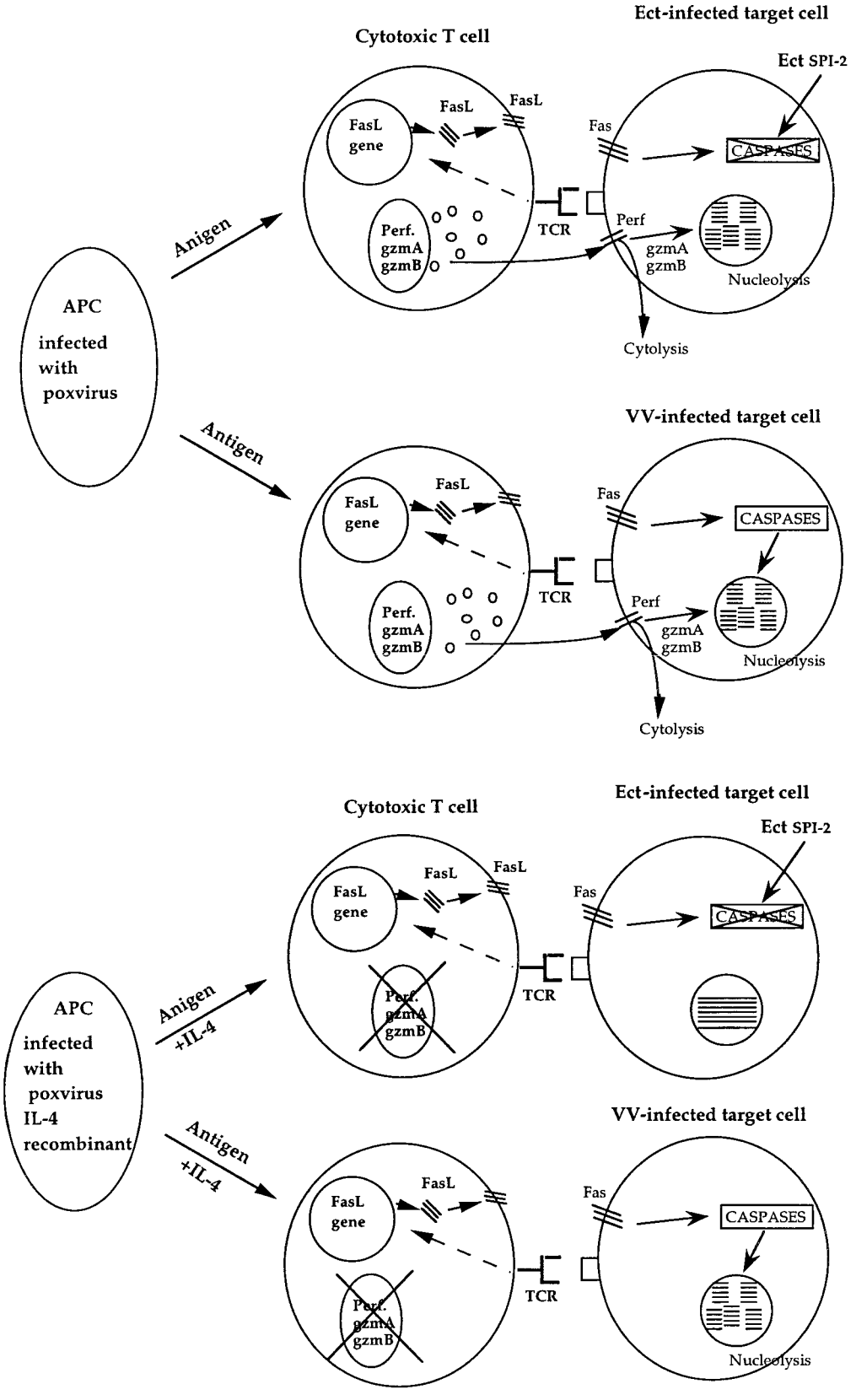

FIG. 1. Schematic representation of poxvirus-infected target cell killing by Tc cells. (Top) Antigen presentation, in the absence of IL-4, leads to activation of Tc cells capable of lysing target cells by both the perforin (exocytosis) and the Fas pathways. Target cells infected with Ect are lysed via the perforin pathway only, as SPI-2 of Ect inhibits killing via the Fas pathway. VV-infected targets are susceptible to lysis by both pathways, as VV-encoded SPI-2 does not interfere with the Fas pathway. (Bottom) Presentation of IL-4 during T-cell activation leads to the generation of Tc cells deficient in the perforin pathway (3). VV-infected targets can be lysed via the Fas pathway. Ect-infected targets are refractory to Tc cell attack.

encoded serin protease inhibitor or serpin, first identified in cowpox virus as cytokine response modifier A (crmA) or SPI-2 (19). All orthopoxviruses encode serpins with SPI-1, -2, or -3 homology between Ect, cowpox virus (CPV), VV, rabbitpox virus (RPV), and variola virus of 92 to 97% (21). Yet despite such a high sequence conservation, VV, in contrast to Ect, CPV, and RPV, does not inhibit Tc cell-mediated lysis by either the exocytosis or the Fas cytolytic pathway (16). Thus, an IL-4-induced increase in Fas-mediated cytotoxicity will be effective in VV infections but not in Ect infections. This may explain the more dramatic increase in virulence of Ect (8) over VV (1) when IL-4 is expressed. Figure 1 dispicts this scenario in schematic form. One prediction would be that the Ect-IL-4 virus would be much less virulent if the SPI-2 gene were deleted. It is not known if variola virus infection alters target susceptibility to Tc cell lysis as is the case for Ect or CPV. Therefore it is also not known if insertion of IL-4 into variola virus would create a much more virulent virus for humans like what happened with Ect for mice and as such be a potential target as a biological warfare agent.

Thus, available evidence fully predicted that (i) Ect-IL-4 recombinant virus would be more virulent and that antibody responses would not be enhanced and that (ii) increased virulence is in part also due to a switch by Tc cells from exocytosis-mediated cytolytic mechanisms to Fas-mediated killing, which is not executable in Ect-infected cells.

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