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Comment

Cytotoxic T lymphocyte memory: role in cross-protective immunity against influenza?

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MHC class I restricted $CD8^+$ cytotoxic T lymphocytes (CTL) have been shown to play a role in the clearance of several virus infections and thus in the prevention of disease symptoms they may cause[1–9].

Although the induction of protective CTL responses directed to influenza virus has been the subject of several studies (see *Table 1* for review), a role of these cells in the protection from subsequent influenza virus infections is controversial. Despite the existence of conserved CTL epitopes present in e.g. the nucleoprotein (NP)[10,11] of influenza virus strains that infect humans in subsequent seasons, there is apparently little or no protective effect mediated by CTLs directed against these epitopes[12]. For this reason it is argued that predominantly the presence of virus neutralizing (VN) antibodies with the relevant specificities for the hemaglutinin (HA) and the neuraminidase (NA) membrane glycoproteins of the actually circulating influenza virus strains is protective: protection against infection proved to be correlated with VN serum antibody titers, induced by natural infection, vaccination or passive transfer[13]. In animal models it was demonstrated that CTL responses are not a requirement for the recovery from influenza infections (Eichelberger *et al.*, 1991[33]; Scherle *et al.*, 1992[34]) and that vaccination with recombinant vaccinia viruses (rVV) expressing the NP or other internal proteins, resulted in virus-specific MHC class I restricted CTL responses but did not protect against a lethal challenge with influenza virus A/PR/8/34 (H1N1)[14–16]. However, in some cases a protective effect could be observed after adoptive transfer of CTLs of this specificity[8,16,17]. Immunization with rVVs expressing the HA or NA proteins led to the induction of virus-neutralizing antibodies and complete protection from challenge infection. Furthermore it was shown that in β2m- knockout mice, which lack

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Table 1 Induction of protection through CTL responses by influenza subunit preparations

Protein	Virus	Presentation form	Species	Immune response demonstrated	Protection	Reference
NP	X/31 H3N2	Adjuvant: none	Balb/c mice (H-2^d)	CTL	Yes	Wraith *et al.*[25]
rNP	A/PR/8/34 H1N1	Adjuvant: $Al(OH_3)$	Balb/c, B10.A (5R) mice	Th	Yes	Tite *et al.*[18]
rNP	A/PR/8/34 H1N1	*Salmonella typhimurium*	Balb/c mice	Th	Yes	Tite *et al.*[19] Brett *et al.*[26]
rNP	A/PR/8/34 H1N1 A/HK/68 H3N2	DNA	Balb/c mice	CTL	Yes	Ulmer *et al.*[24]
rNP	A/PR/8/34 H1N1	Vaccinia virus	B6 and ß2m⁻ mice (H-2^b)		No	Epstein *et al.*[15]
HA		Vaccinia virus	B6 and ß2m⁻ mice	VN	Yes	
NA		Vaccinia virus	B6 and ß2m⁻ mice	VN	Yes	
PA, NS1, NS2, PB1, PB2, M1, M2, NP. alone or as a mixture		Vaccinia virus	B6 and ß2m⁻ mice		No	
HA		Vaccinia virus	(old) mice	VN/CTL	Yes	Ben-Yehuda *et al.*[27]
HA		Vaccinia virus-IL-2	nude mice	VN/γIFN	Yes	Karupiah *et al.*[22]
HA	H1/H2	NS1-HA2 fusion protein	Balb/c mice	CTL	Yes	Kuwano *et al.*[28]
HA	H1H1	Vaccinia virus	CBA/h mice	VN	Yes	Andrew *et al.*[16]
NP	H1N1	Vaccinia virus	CBA/h mice	ND	No	Andrew *et al.*[16]
NP 147-155	H1N1	Vaccinia virus	Balb/c mice	Pulmonary CTL	No	Lawson *et al.*[14]
NP	H1N1	Vaccinia virus	Balb/c mice	Pulmonary CTL	No	Lawson *et al.*[14]
NP	H7N1	Vaccinia virus	C57 BL/6 (H-2b) CBA/J (H-2k) mice	CTL	No	Stitz *et al.*[29]
NP	H7N1	Cell associated	idem	CTL	No	Stitz *et al.*[29]
NP	H2N2	ISCOM	Balb/c and C3H (H-2k) mice	No CTL	Partially	Weiss *et al.*[30]
NP	H5N2/H5N8	Fowl pox	chicken		No	Webster *et al.*[31]
NP	H1N1	Vaccinia virus	outbred ddY mice	CTL in Balb/c	No, reduced pulmonary virus titers d7 p.i.	Endo *et al.*[32]

$CD8^+$ CTL, HA and NA protein expressing rVVs induced protection that was fully mediated by VN serum antibodies. However, it should be realized that in most of the studies which failed to induce protective immunity by mechanisms other than VN antibodies, rVVs were used as expression system for viral proteins like NP. In other experiments using purified NP or recombinant NP as immunogens, either presented as soluble protein or by *Salmonella typhimurium* as a vector, protection could be induced. With *Salmonella* as an antigen delivery system for NP or with recombinant NP in $Al(OH)_3$, strong T helper (Th) cell responses were induced. No direct association of protection with virus-specific MHC class I restricted CTLs was demonstrated[18, 19], probably since antigens presented in this way are highly inefficient in entering the endogenous pathway of antigen processing[20, 21]. A mechanism underlying this protection may be the accelerated induction of VN antibodies and CTLs as a result of strong T helper cell responses after infection with the challenge virus. However, since the immunized mice in these exeperiments were also protected from challenge with an influenza B virus, the action of non-specific antiviral mechanisms such as the production of IFNγ could not be excluded[19]. Studies in nude mice showed that a rVV coexpressing IL-2 along with HA may confer protection in mice in the absence of T cells and suggested a role for specific IgM antibodies, natural killer cell activity and IFNγ[22].

Recently, alternative systems have been explored for the presentation of NP to the immune system including nucleic acid vaccination: plasmid DNA, which encodes a foreign protein under control of a eukaryotic promotor sequence, was directly injected into muscle tissue of recipient mice. Inoculation of mice with influenza NP encoding DNA, resulted in decreased virus titers in the lung, inhibited mass loss and increased survival rates not only after a homologous challenge with influenza A/PR/8/34 (H1N1), but also after a heterologous challenge with influenza virus A/HK/68 (H3N2). In parallel experiments neither the passive transfer of NP-specific antibodies, nor the immunization with purified NP could confer protection. In the NP-DNA inoculated mice, NP-specific CTL were demonstrated which were absent in mice immunized with the purified protein.

The observation that humans may develop influenza upon infection with newly emerging virus strains and that mice are not protected against a virus challenge after vaccination with rVV expressing the NP protein, in spite of the induction of CTL responses, suggests an inadequate induction of CTL memory.

Recently it was shown that for the induction of protective cytotoxic T cell memory towards lymphocytic choriomeningitis virus (LCMV) infection, the persistence of viral antigen was required[23]. LCMV-specific long-term CTL memory was only maintained in persistent LCMV infection. Vaccination with rVVs expressing LCMV proteins resulted in a poor CTL memory, which correlated with the rVV dose and depended on the LCMV protein expressed. Adoptive transfer experiments with spleen cells from immunized mice confirmed that for the maintenance of CTL memory the presence of viral antigen was essential. These findings are in contrast to results recently obtained in adoptive transfer experiments, which were performed with irradiative recipient mice (Mullbacher, 1994[35]; Hou *et al.*, 1994[36]), suggesting that thus acquired influenza virus specific CTL memory can be maintained in the absence of antigen. The protective effect of these CTLs was, however, not studied by challenge infection.

Therefore we speculate that antiviral protection mediated by memory CTL is short-lived when induced by non-persistent infection with, for example, influenza virus. Similarly, vaccination with rVV expressing the relevant influenza proteins or live attenuated influenza vaccines may only induce short-lived CTL responses in the absence of persisting antigen. In contrast the persistent low level *in vivo* expession of NP after inoculation with NP-DNA[24], may lead to protective immunity mediated by specific CTL.

Therefore it may be expected that if indeed the development of future influenza vaccines should take advantage of the existence of conserved CTL epitopes[10, 11], resulting in the induction of inter- and intratypic cross-protection, they should be based upon strategies leading to the persistent exposure of the immune system to the relevant viral antigens.

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