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Exquisite specificity of adoptive immunization in arenavirus-infected mice

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Summary

Lymphocytic choriomeningitis virus (LCMV)-infected mice can be adoptively immunized with T cells from immune mice sharing MHC compatibility in H-2K or D, suggesting direct cytotoxic effects of T cells in vivo. However, T cells, upon recognition of an appropriate target, secrete lymphokines which may be capable of mediating antiviral effects nonspecifically. In this report we show that LCMV-immune cells reduced LCMV spleen titers in mice infected with LCMV alone or with LCMV and Pichinde virus (PV), but had no effect on PV titers in these mice or in mice infected with PV alone. Titers of PV were reduced by PV-immune cells transferred into mice infected with PV alone or with PV and LCMV, while LCMV titers were not altered. PV and LCMV antigens were shown by fluorescence microscopy to be in proximity in the spleen prior to cell transfer. These data suggest that adoptive immunization against these arenaviruses involves direct cytotoxicity or an extremely localized effect of nonspecific soluble factors elaborated following antigen recognition, rather than generalized nonspecific antiviral effects of a more systemic nature.

lymphocytic choriomeningitis virus; Pichinde virus; adoptive immunization; cytotoxic T lymphocytes

Introduction

The arenaviruses lymphocytic choriomeningitis virus (LCMV) and Pichinde virus (PV) cause acute infections in mice characterized by potent virus-specific cytotoxic T lymphocyte (CTL) responses. These viruses have structural similarities and serologically crossreact at the level of the nucleocapsid protein but not at the level of the

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envelope glycoproteins [1]. Mice acutely infected with LCMV receiving virus-immune splenocytes at early stages of infection rapidly clear virus in the spleen and other organs [2,3]. This process is called adoptive immunization [2]. Zinkernagel and Welsh [2] showed that adoptive immunization of mice infected with LCMV required θ-bearing lymphocytes from LCMV-immune mice syngeneic with recipient mice in the K or D but not the I region of the H-2 locus of the major histocompatibility complex (MHC). Since CTL have similar K/D recognition patterns [4], it was suggested that the adoptive immunization may be mediated by CTL which lyse the virus-infected target cells in vivo [2]. Recently Byrne and Oldstone have shown that LCMV-specific cloned cytotoxic T cell lines also can mediate adoptive immunization [5].

However, upon recognition of an appropriate target, T cells secrete a number of soluble factors including immune interferon (IFN-γ) [6], macrophage migration inhibition factor (MIF) [7], and lymphotoxins [8]. IFN-γ has been shown to markedly inhibit virus synthesis [9], to activate natural killer cells [10], and to activate macrophages [11] (macrophage activation factor (MAF) is now considered to be the same as IFN-γ [12]). All these factors can contribute to the resistance to various virus infections. It is possible, therefore, that the antiviral effect seen during adoptive immunization is not due to direct and specific cytotoxicity, but to nonspecific events that follow triggering of the T cell by antigen recognition. In this report we transfer immune splenocytes into mice infected with two arenaviruses and show that the antiviral effect mediated by adoptive immunization is virus-specific.

Experimental

The rationale for the following experiments was to infect mice simultaneously with LCMV and PV, to adoptively transfer into those mice immune spleen cells directed against one of the viruses, and to determine whether the resulting inhibition in virus synthesis was selective for only one virus. Dual infections in 6–12-week-old C3H/St male mice (West Seneca Farms, West Seneca, NY) were established by injecting them with 8×10^4 pfu LCMV i.p. and 1×10^6 pfu PV i.v. To discriminate between the two viruses in the dually infected mice, PV was titrated by Vero cell plaque assays following neutralization of LCMV with guinea pig anti-LCMV serum. LCMV was titrated in the presence of PV by staining plaque assay plates with iodophenyl nitrophenyl phenyl tetrazolium chloride (INT; Sigma), a dye that stains uninfected and PV-infected cells but not LCMV-infected cells [13]. Thus, LCMV plaques appear clear on a stained background.

Virus-immune spleen cells were from mice infected 7 days previously by intraperitoneal (i.p.) inoculation of LCMV (8×10^4 pfu) or PV (1×10^6 pfu). Leukocytes were prepared by treatment of splenocytes with 0.83% NH_4Cl to lyse erythrocytes. Table 1 shows that these immune cells selectively lyse their respective virus-infected target cells in vitro, as demonstrated previously [14].

Immune or control spleen cells were inoculated i.v. into virus-infected recipient mice 24 h post-infection, as described previously [2]. Spleens from the recipient mice were removed for virus titration 48 h following cell transfer. A reduction in spleen virus titer

TABLE 1

In vitro cytotoxic specificity of virus-immune spleen cells

Spleen cells	% specific ^{51}Cr release*		
	L929	L929 + PV	L929 + LCMV
Normal C3H	-1.0	0.3	0.2
LCMV-immune	3.4	0.7	32.0
PV-immune	1.1	33.0	4.1

Target cells were histocompatible uninfected L929 cells or L929 cells infected with PV or LCMV. Sodium ^{51}chromate (New England Nuclear, Boston, MA)-labeled targets (10^4) were mixed with normal or day 7 virus-immune splenocytes (5×10^5) in 0.2 ml Eagle's minimal essential medium (MEM) (GIBCO, Grand Island, NY) supplemented with antibiotics, glutamine and 10% heat-inactivated fetal bovine serum in round-bottom microtiter plates (Costar, Cambridge, MA). Incubation was at 37°C in a humidified atmosphere of 5% CO_2 and 95% air for 6 h. Media and Nonidet P-40 (NP-40) were added to wells to determine spontaneous and maximum lysis, respectively. The assays were done in quadruplicate with standard errors less than 10% of mean values.

* [(Test cpm - MEM cpm)/(NP-40 cpm - MEM cpm)] × 100.

compared to controls is indicative that the adoptive immunization was successful. As seen in Table 2, reduction in titers in mice infected with only one virus was, as suspected, mediated only by the relevant virus-immune splenocytes. LCMV-immune spleen cells adoptively immunized singly infected mice to LCMV but not to PV (Table 2A), whereas PV-immune spleen cells adoptively immunized only to PV but not to LCMV (Table 2B). The groups of prime interest, however, were those infected with both LCMV and PV. Adoptive immunization of dually infected mice with LCMV-immune splenocytes reduced the titers of LCMV by over 95% but had no effect on PV titers (Table 2A). Similarly, adoptive immunization of dually infected mice with PV-immune splenocytes reduced PV titers but not LCMV titers (Table 2B). In these experiments, therefore, the specificity of adoptive immunization against arenavirus infection in vivo (Table 2) correlates with the specificity of virus-immune CTL recognition patterns in vitro (Table 1).

To examine the possibility that the fine specificity of the immunizing cells was due to low titers of the two viruses growing in widely separated regions of the spleen at the time (day 1 post-infection) of adoptive transfer, recipient mice were lethally irradiated (1000 rad) to suppress their own immune response, then dually infected with LCMV and PV. These mice were adoptively immunized with LCMV-immune cells on day 3 post-infection and spleen virus titers were determined 2 days after cell transfer. The data in Table 3 again demonstrate the specific reduction of LCMV titers within the spleen following LCMV-immune cell transfer with no effect observed on the heterologous PV titers.

In a parallel experiment, the spleens of lethally irradiated, dually infected mice were obtained 3 days post-infection and processed for virus-specific immunofluorescent staining. Serial four micron frozen sections were stained with LCMV- or PV-immune guinea pig sera. Side-by-side comparison of these sections (Fig. 1) indicated that both viruses were indeed growing in very close proximity to one another at the time of adoptive immunization.

TABLE 2

Specificity of arenavirus-immune spleen cells in adoptively immunized mice

Virus inoculated	Spleen cells transferred	Virus titer $\log_{10}$ pfu/spleen	
		PV	LCMV
(A) LCMV	Normal	-	4.9 ± .38
	LCMV-immune	-	2.4 ± .10*
PV	Normal	5.2 ± .05	-
	LCMV-immune	5.4 ± .08	-
LCMV + PV	Normal	5.5 ± .05	4.1 ± .82
	LCMV-immune	5.5 ± .18	2.8 ± .18**
(B) LCMV	Normal	-	4.4 ± .42
	PV-immune	-	4.6 ± .49
PV	Normal	4.4 ± .18	-
	PV-immune	3.2 ± .76**	-
LCMV + PV	Normal	4.6 ± .13	3.7 ± .44
	PV-immune	3.0 ± .11*	3.5 ± .21

Mice ($n = 4$ per group) received 5 (Expt. A) or 8 (Expt. B) $\times 10^7$ normal or day 7 virus-immune spleen cells i.v. 24 h after inoculation of 8×10^4 pfu LCMV i.p., 10^6 pfu PV i.v., or both. Spleens were removed 2 days later and homogenized in MEM. The homogenate was clarified by centrifugation at 2000 rpm for 20 min at 4°C and virus plaqued on Vero cell monolayers. Titer significantly different from recipients of normal cells: * $P<0.001$, ** $P<0.05$.

TABLE 3

Adoptive immunization of lethally irradiated, dually infected mice

Spleen cells transferred	Virus titer $\log_{10}$ pfu/spleen	
	PV	LCMV
Normal	3.4 ± .15	4.0 ± .50
LCMV-immune	3.4 ± .15	2.7 ± .32*

Mice ($n = 4$ per group) were irradiated (1000 rad, 125 rad/min, ^{137}Cs source) and infected with 8×10^4 pfu LCMV i.p. and 10^6 pfu PV i.v. On day 3 post-infection, mice received 5×10^7 normal or immune spleen cells i.v. Spleens were removed 2 days later and processed for virus titers as described in Table 2.

* Virus titer significantly lower than recipients of normal cells ($P<0.05$).

Previous studies had shown that vaccinia-immune splenocytes failed to immunize against LCMV infection, but they were transferred into mice only infected with LCMV and were unlikely to be stimulated in that environment [2]. Our experiments, however, were designed to ensure that the transferred immune T cells were reactive with antigens in the recipient mice. Thus, under the present conditions where transferred virus-immune splenocytes were stimulated by the antigen to which they had been primed, no nonspecific antiviral effect was evident.

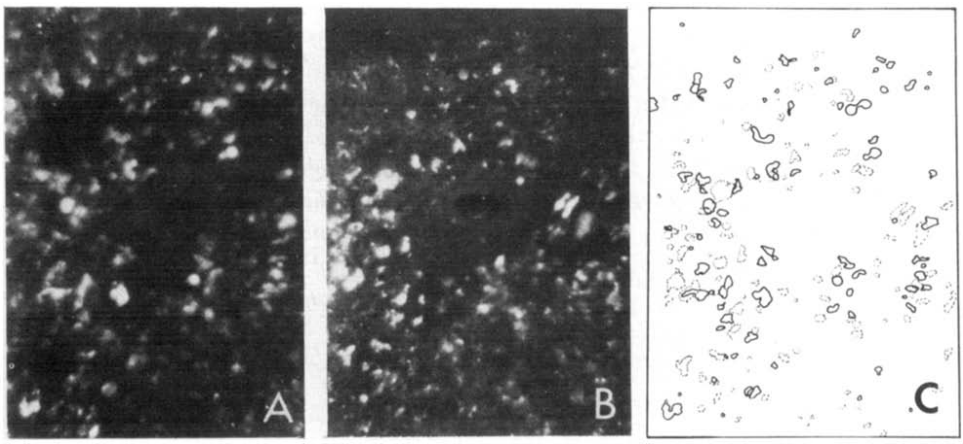

Fig. 1. Localization of LCMV and PV antigens in the spleens of dually infected mice. Irradiated (1000 rad) mice were infected with LCMV and PV. Spleens were removed 3 days postinfection and frozen. Four micron serial sections of frozen spleens were fixed in ether and ethanol, air-dried, then incubated with either LCMV-specific or PV-specific immune guinea pig serum. The sections were washed then stained with rhodamine-conjugated goat anti-guinea pig serum. Neither virus-specific antiserum stained uninfected spleens or spleens singly infected with the heterologous virus (not shown). Panel A (LCMV antigen) and panel B (PV antigen) represent the same area of adjacent sections. Panel C is a composite drawing (LCMV antigen, solid lines; PV antigen, dashed lines) made by superimposing panels A and B. Original magnification, × 400.

We found this exquisite specificity in adoptive immunization to be somewhat surprising for several reasons. One might expect that virus in the vicinity of other viruses to which the immune response is directed may be affected by non-specific lymphokines. Lehmann-Grube and coworkers have shown that cytotoxic Lyt-2,3+ cells alone cannot transfer immunity in the LCMV model. Rather, Lyt-2,3+ cells are apparently required [15]. This would suggest that factors other than cytotoxicity may be of importance. On the other hand, the Lyt-1+ phenotype may simply be required for maintenance of the Lyt-2,3+ population in vivo. Zinkernagel and Welsh showed that only K or D compatibility is required for adoptive immunization in the LCMV system [2], and Byrne and Oldstone [5] have demonstrated the ability of LCMV-specific, H-2-restricted cloned CTL to adoptively immunize histocompatible LCMV-infected mice. These results indicate that cells with cytotoxic T cell phenotypes and recognization patterns mediate antiviral effects in vivo but still do not discriminate between direct in vivo cytotoxic effects and potential nonspecific antiviral effects mediated by soluble factors.

A recent report by Lukacher et al. [16] using an influenza virus system has demonstrated specific virus reduction in the lungs of dually infected mice following adoptive transfer of anti-influenza CTL clones. In mice infected with two influenza A subtypes, a subtype-specific CTL clone reduced virus titers only of the recognized subtype, while a subtype cross-reactive clone lowered titers of both subtypes. The in vivo reactivity of these clones mirrored their in vitro cytolytic reactivities. This work, like our present study, is consistent with a highly localized effect of the T cells, such as direct cytotoxici-

ty. However, prior success in the influenza virus CTL clone adoptive transfer model has been highly variable, with some clones providing in vivo protection [17] while others do not [18], despite showing in vitro cytolytic specificity. Thus, the inhibition of virus replication seen in the adoptive transfer models using cloned CTL lines may represent specificity phenomenon peculiar to the individual clones used. Our data obtained following adoptive transfer of immune whole spleen cells may more closely reflect the normal antiviral activity of the specific immune response of the infected host. Our results thus support the concept that T cell-dependent adoptive immunization against arenavirus infections involves either direct cytotoxic effects by immune splenocytes, or else a highly localized and restricted nonspecific antiviral effect following antigen recognition.

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