Brief Definitive Report

VIRUS-TRIGGERED IMMUNE SUPPRESSION IN MICE CAUSED BY VIRUS-SPECIFIC CYTOTOXIC T CELLS

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Virus infection may cause transient or long-lasting immune suppression, the pathogenesis of which has been explained by several possible mechanisms (1–5). (*a*) Virus is directly cytopathic for lymphocytes and possibly for antigen-presenting cells, macrophages, and astrocytes. (*b*) Viral antigens on cell surfaces may enhance cell fusion thereby causing destruction of cells. (*c*) Soluble or cell-bound viral antigens may be immunosuppressive. (*d*) Antiviral antibodies or virally induced autoantibodies may cause elimination of lymphocytes or other mononuclear cells (6). (*e*) Virus-specific cytotoxic T cells may destroy virus-infected lymphocytes, dendritic cells, macrophages, etc., a possibility for which circumstantial evidence exists (7).

This study attempts to analyze pathogenetic mechanisms of virally triggered immune suppression in a mouse model of immune suppression caused by infection with lymphocytic choriomeningitis virus (LCMV) (2, 4, 5, 8–10). Infection of mice with LCMV strongly suppressed the T-independent IgM and a strictly T help–dependent IgG immune response against a second infectious agent (11). This suppression was observed in immunocompetent adult mice infected with LCMV, but not in tolerant LCMV carrier mice, or mice infected with LCMV that were treated with an mAb to eliminate cytotoxic T $CD8^+$ cells. Thus, the immune suppression in this model is not caused by LCMV itself nor by IFN induced by it, but rather by the T $CD8^+$–dependent immune response against LCMV.

Materials and Methods

Mice. Inbred C57BL/6 (H-2^b), DBA/2 (H-2^d), and colony-bred ICR (H-2^q) *nu/nu* and +/+ mice were purchased from the Institut für Zuchthygiene, Tierspital, University of Zürich. C57BL/6 *nu/nu* mice were a gift from the Institut für biologisch-medizinische, Forschung AG, Füllinsdorf, Switzerland. Mice were 6–16 wk old.

Virus and Immunization. The various isolates of LCMV had the following origin: WE was from Dr. F. Lehmann-Grube, Hamburg, Federal Republic of Germany, and ARMSTRONG (ARM) was from Dr. M.B.A. Oldstone, Scripps Clinic, La Jolla, CA (5, 10). LCMV was titrated in vivo intracerebrally or in the footpad to determine the mean infectious dose for 50% (ID) of the mice. Seeds of VSV-NJ (Pringle isolate) have been obtained from Dr. D. Kolakovsky, University of Geneva, Switzerland, and were grown on BHK21 cells infected with low multiplicity (12). Mice were usually infected with 0.2 ml i.v.

This work was supported by Swiss National Foundation Grants SNF 3.259-0.85 and by the Kanton Zürich.

Determination of Neutralizing Antibody. The standard neutralization assay was used as described previously (12). In brief, serial twofold dilutions of heat-inactivated sera were mixed with equal volumes of virus containing 50–80 plaque-forming units (pfu) in 100 μl and incubated at 37°C for 90 min. The dilution of the serum resulting in 50% reduction of plaques was determined on Vero cell monolayers grown in a 24-well plate (model 3024; Costar, Cambridge, MA). To determine IgG titers, serum was pretreated with 0.05 M 2-ME.

Antibodies. The rat IgG 2b mAbs YTS 169.4 (anti-Lyt 2) and YTS 191.1 (anti-L3/T4) were prepared and used as described elsewhere (13).

Detection of VSV and IFN In Vivo. Replication of VSV (11) in vivo was determined in 10% homogenates of various organs; IFN levels were measured as described elsewhere (14).

Results and Discussion

No Immune Suppression in Mice Tolerant to LCMV or in LCMV-infected Nude Mice. Mice infected with LCMV-WE showed signs of a long-lasting immune suppression of antibody responses against a second virus infection. When C57BL/6 or ICR mice infected with 10^5 ID of LCMV were subsequently infected with vesicular stomatitis virus (VSV) 8–30 d later, they made a reduced (C57BL/6) (Table I) or no (ICR) (Table II) T cell–independent IgM response and failed to mount a T $CD4^+$ help-dependent anti-VSV IgG response. When C57BL/6 mice from a LCMV-carrier colony that are tolerant to LCMV (at least at the T cell level due to transplacental infection with this noncytophathic, or poorly cytopathic virus before maturation of T cell competence [5, 8, 10]) were

TABLE I

Capacity of Infected C57BL/6 +/+ or nu/nu Mice or of LCMV-carrier Mice to Produce Neutralizing Anti-VSV Antibodies

	Primary infection* (days before VSV)	Treatment	Neutralizing anti-VSV titers			
			Day 4		Day 8	Day 12
			IgM	IgG	IgG	IgG
Exp. 1:						
C57BL/6 +/+	LCMV(−30 d)	—	1,280	<80	160	160
	LCMV (−8 d)	—	640	<80	<80	<80
	—	—	2,560	<80	5,120	20,480
Exp. 2:						
C57BL/6 +/+	LCMV (−8 d)	—	640	<80	<80	<80
	—	—	10,240	<80	5,120	20,480
	LCMV (−8 d)	anti-CD8‡	2,560	<80	10,240	20,480
	—	anti-CD8‡	2,560	<80	5,120	20,480
	LCMV	carrier§	10,240	<80	2,560	20,480
C57BL/6 *nu/nu*	LCMV	—	10,240	<80	<80	<80
	—	—	10,240	<80	<80	<80

* C57BL/6 +/+ (Institut für Zuchthygiene; Tierspital Zürich; Switzerland) and C57BL/6 *nu/nu* mice (Institut für Medizinische Forschung; Füllinsdorf; Switzerland) were infected with 10^5 ID of LCMV-WE 8 d before the inoculation of VSV-NJ. After infection with 10^6 pfu VSV-NJ, they were bled on days, 4, 8, and 12, thereafter, indicated values are mean of IgM and IgG titers of three individual serum samples. If not indicated otherwise, measurements did not differ by more than one titration step from the respective mean.

‡ Mice were injected with rat anti–mouse CD8 mAb (YTS 169.4 [13]), a monoclonal rat anti–mouse CD4 (YTS 191.1 [13]) 6, 5, and 4 d before the infection with VSV-IND.

§ Virus carrying offsprings from persistently infected mothers.

TABLE II

Capacity of LCMV-infected ICR +/+ or nu/nu Mice to Produce Neutralizing Anti-VSV Antibodies: Effects of Treatment with Anti-CD8 or of Adoptive Transfer of LCMV-immune Lymphocytes

	Primary infection	Treatment	Anti-VSV serum neutralization titers*				
			Day 4		Day 8	Day 12	
			IgM	IgG	IgG	IgM	IgG
ICR +/+	LCMV	—	<80	<80	<80	ND	<80
	—	—	2,560	<80	1,920	ND	5,120
	LCMV	anti-CD8	1,290	<80	640	ND	5,120
ICR *nu/nu*	LCMV	—	1,280	<80	<80	160	<80
	—	—	2,560	<80	<80	160	<80
	LCMV	Transfer of‡ LCMV-immune lymphocytes($CD4^-$)	<80§	<80			
	LCMV	Transfer of Vaccinia-immune lymphocytes(CD4)	1,280	<80			

* Experimental procedures are given in Table I.

‡ Mice were injected with 1 X 10^8 spleen lymphocytes from 8-d LCMV-WE or 6-d Vaccinia virus–immune ICR +/+ mice at the same time as the VSV-NJ infection. LCMV immune lymphocytes were from mice treated with anti-CD4 (YTS 191.1) on day −1, 1, and 3.

§ Represents the mean of three mice; none of the animals survived day 5.

superinfected with VSV, they made a normal IgM and IgG response. This finding is compatible with previous studies showing more or less normal immune responses in LCMV-carrier mice to a variety of antigens and infectious agents (10, 15). Similarly, nude mice infected with LCMV and thereafter inoculated with VSV mounted a normal IgM response to VSV. Thus, mice connatally tolerant to LCMV (carrier mice) and mice that were unable to mount a T cell response to LCMV (carrier mice and nude mice) were not susceptible to LCMV-induced immune suppression.

Immune suppression caused by LCMV infection of fully T cell–immunocompetent mice could not be caused by IFN induced by LCMV, which could possibly influence the availability of immunogenic VSV antigens, for the following reasons. (*a*) C57BL/6 mice infected with 10^5 ID of LCMV had IFN-α and -β in the blood on day 2 and 4 (1,280–10,000 U/ml) but IFN had dropped below detectable levels by day 6 (<20 U/ml), when VSV was usually injected on day 8–10. (*b*) Nude mice infected with LCMV have IFN-α and -β in their serum (16), but their antibody response to VSV was not suppressed (Table I). (*c*) Normal mice infected with LCMV, but treated with anti-CD8 antibodies, made normal anti-VSV IgM or IgG responses despite the fact that they have been shown to express LCMV-induced levels of IFN-α and -β (13) that were comparable with those found in control mice or mock treated mice. (*d*) VSV was not found to replicate in DBA/2 or C57BL/6 mice in other organs than the spinal cord and/or the brain; at no time could we find plaque-forming VSV in liver, lung, spleen, or kidneys at 6 h, 1, 2, 3, 5, or 7 d after injection in either normal or LCMV-preinfected mice (data not shown). (*e*) LCMV infection suppressed IgG responses to UV-inactivated or formaldehyde-inactivated VSV (11), further indicating

that VSV replication was not necessary and that IFN effects thus could not explain the results. Therefore, immune suppression apparently is not caused by LCMV itself or IFN induced by it, but rather by the anti-LCMV immune response.

Experiments on the Role of Cytotoxic T Cells in LCMV-triggered Immunosuppression. C57BL/6 (Table I) or ICR (Table II) mice infected with LCMV but then treated with a rat anti–mouse CD8 mAb (YTS 169.4; anti-Lyt 2) (13), before they were superinfected with VSV, mounted a normal IgM and IgG response to VSV. This anti–CD8 mAb had been shown earlier to eliminate T $CD8^+$ but not T $CD4^+$ cells (13) in vivo; and prevented generation of LCMV-specific cytotoxic T cells. Neither an irrelevant rat mAb (not shown) nor treatment of LCMV-infected mice with an anti-CD4 antibody relieved the suppression of T $CD4^+$–independent IgM anti-VSV antibodies; because of the direct effect of the latter antibody on T $CD4^+$ helper cells (13), its effect on suppression of IgG response by LCMV infections could not be evaluated. In a second experimental approach, LCMV-infected ICR nude mice were given LCMV immune spleen cells from mice pretreated with anti-CD4 antibody (to prevent T helper cells and IgG antibodies to be generated) on the same day as the VSV infection; IgM anti-VSV responses measurable on day 4 were reduced when compared with recipients of Vaccinia virus–immune spleen cells. These experiments suggest that anti-LCMV T $CD8^+$ cells were involved in causing the observed immune suppression.

Efficient cell-mediated immunity is crucial for the recovery of a host from many acute viral infections (5, 10); cytotoxic T cells are apparently involved in this process by destroying infected host cells to prevent virus replication. Although essential to overcome infections with cytopathic viruses, this immunological effector pathway may cause more cell and tissue damage than necessary in the case of infection with noncytopathic viruses. In these latter infections, the balance between virus spread and T cell immune response determines whether either virus elimination (protection) or cell and tissue damage (immunopathology) predominate or whether a virus carrier state (no virus elimination, no cell and tissue damage) results. Acute hepatitis B, chronic aggressive hepatitis or hepatitis carrier status in humans (17) and acute LCM disease, chronic LCM-wasting disease, or LCMV carrier status in mice (5, 8, 10) are examples for these varying equilibrium conditions between virus and immune response. Similar considerations may explain some aspects of HIV infection of man, and we should like to postulate that HIV-induced AIDS, similar to LCMV-induced immune suppression, may well be an extreme form of an immunopathological disease where one subpopulation of effector lymphocytes (T $CD8^+$) destroys virus-infected APC and macrophages or T cell subpopulations (e.g., T $CD4^+$). There exists evidence for HIV (3), but recently also for LCMV (18), that T helper cells (T $CD4^+$), but not killer T $CD8^+$ cells, are infected by the virus; also like HIV (3, 6), LCMV infects, besides many other cells, macrophages and dendritic cells very efficiently (5, 10). Whether antiviral T $CD8^+$ cells destroy infected lymphocytes and mononuclear cells or APC involved in immune responses in vivo, remains to be established. The report that T $CD8^+$ cells can control HIV production in vitro (7) and that HIV immune T $CD8^+$ cells destroy infected macrophages efficiently in vitro (6) are compatible with the idea.

In conclusion, the presented evidence suggests that virus-specific cytotoxic T cells may act as suppressor T cells and that immune suppression in LCM is caused by a T cell–mediated immunopathology. It is conceivable that a similar pathogenesis may be responsible for immune suppression and AIDS caused by HIV infection in humans.

Summary

Normal mice infected with 10^5 infectious doses of lymphocytic choriomeningitis virus (LCMV, WE isolate) generated a reduced or no T cell–independent IgM and/or T cell–dependent IgG response to a subsequent vesicular stomatitis virus Indiana (VSV-IND) injection; this transient immune suppression lasted for weeks to months. Connatally infected LCMV-carrier mice or acutely infected T cell–deficient nude mice had normal anti-VSV IgM and IgG or IgM responses respectively. LCMV-infected nude mice transfused with helper cell–depleted LCMV-specific immune spleen cells were immunosuppressed. Normal mice infected with LCMV but treated with a rat anti-CD8 mAb (that had been shown previously to eliminate cytotoxic T cells in vivo) and then infected with VSV exhibited a normal anti-VSV IgM and IgG response. Since no IFN-α or -β was detected on, or after, day 6 of LCMV infection, neither LCMV alone, nor IFN induced by it caused the observed immune suppression; the presented evidence suggests that LCMV-immune $CD8^+$ T cells were responsible for it. It is conceivable that a similar pathogenesis where virus-specific cytotoxic T cells may destroy virus-infected cells essentially involved in an immune response (APC, T helper cells, etc.) may be involved in other virally triggered immune suppression or in AIDS.

We thank Dr. H. Waldmann for the seeds of the hybridomas YTS 169.4 and 191.1, the Swiss National Science Foundation, and the Kanton Zürich for support.

Received for publication 6 October 1987 and in revised form 17 February 1988.

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