

Brief Definitive Report

VIRALLY INDUCED MODULATION OF MURINE IgG ANTIBODY SUBCLASSES

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Murine IgG antiviral antibodies elicited by infection have recently been shown to belong predominantly to the IgG2a subclass (1). This isotypic distribution of antiviral antibodies contrasts sharply with that of antisoluble protein or anticarbohydrate antibodies, which are usually restricted to the IgG1 and IgG3 subclasses, respectively (1-3). Such an isotypic bias of antiviral antibodies could be due to intrinsic biochemical characteristics of viral antigens or, alternatively, to regulatory mechanisms elicited by the infectious process itself. To address this question, we analyzed the effect of infection on the isotypic pattern of antibodies not directed against the viral antigens. Our results indicate that viruses can influence the subclass distribution of IgG antibodies produced in the course of the infection, irrespective of their specificity.

Materials and Methods

Mice. CBA/Rij and 129/Sv mice, bred at our institute by Dr. G. Warnier, were maintained in specific pathogen-free conditions and used when 6-10 wk old.

Viruses. Infections were performed as described in (1). In addition, K virus (a gift of Dr. W. P. Rowe, Bethesda, MD, No. 3134/KS) and EDIM virus (a gift of Dr. T. H. Flewett, Birmingham, UK) were used. For the absorption experiments, pooled sera from infected mice were diluted 100-fold in PBS (120 mM NaCl, 5 mM Na₂HPO₄, 3 mM KH₂PO₄, pH 7.2) containing 2% FCS and incubated for 2 h at 4°C with serial doses of purified virus or with buffer only. Viral particles were then removed from sera by centrifugation through a 15% sucrose cushion (35,000 rpm for 90 min in an SW41 rotor, Beckman Instruments, Inc., Palo Alto, CA) and remaining Igs were measured in supernatant by ELISA.

Immunoglobulin Determinations. Total IgM and IgA levels were determined by RIA as described previously (4) and total IgG subclass levels were measured by ELISA. For the ELISA, polystyrene plates (Greiner, Nürtingen, FRG) were coated overnight with an anti-mouse IgG3 rat mAb (2E.6, American Type Culture Collection, Rockville, MD) (5 µg/ml in 0.02 M glycine, 0.03 M NaCl, pH 9.2) or with affinity-purified goat antibodies specific for rabbit IgG, followed by rabbit antibodies specific for mouse IgG subclasses (4). After overnight incubation with sera serially diluted in Tris-buffered saline (10 mM Tris, 10 mM merthiolate, 130

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mM NaCl, pH 7.4) supplemented with 5% FCS, a competition was initiated by addition of biotinylated mAbs of the appropriate subclass for 7 h at 37°C. The fixation of labeled antibodies was measured with avidin-peroxydase revealed by orthophenylene diamine. Reference curves were obtained with unlabeled mAbs of each subclass. Antibody IgG subclasses were measured by ELISA as described in (5).

Results and Discussion

Serum levels of total IgM, IgA, and of each IgG subclass were measured 3 wk after infection of CBA/Rij mice with a panel of common viruses. As shown in Fig. 1, a significant hypergammaglobulinemia was detected after infection with several viruses, including adenovirus, reovirus type 3, lactate dehydrogenase-elevating virus (LDV), mouse hepatitis virus (MHV, A59 strain), Sendai virus, and lymphocytic choriomeningitis virus (LCMV). In most cases, this hypergammaglobulinemia was largely restricted to the IgG2a and, to a lesser extent, to the IgG2b subclasses. For instance, after LDV or LCMV infection, a 47–65-fold increase of IgG2a was observed. In contrast, the other Ig isotype levels were usually only slightly modified.

To determine whether the hypergammaglobulinemia induced by viral infection corresponded only to specific antiviral antibodies or was the result of nonspecific

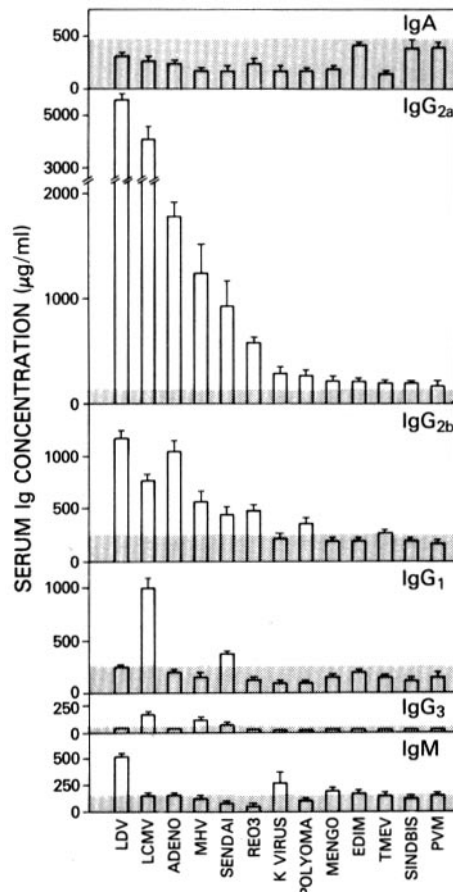


FIGURE 1. Serum Ig levels after viral infection. Groups of 5–19 CBA/Rij mice were infected with a panel of murine viruses (PVM, pneumonia virus of mice; TMEV, Theiler's mouse encephalomyelitis virus), and total Ig levels were measured in individual sera obtained 3 wk later. The hatched zones indicate the values obtained for control mice (mean + 2 SE) and the bars represent the results for infected animals (mean $\pm$ SE).

activation of B lymphocytes, we absorbed sera from infected mice with purified virus. As shown in Fig. 2 for a typical experiment with adenovirus, this treatment eliminated ~85% of specific antibodies. However, >90% of the total serum IgG2a was not absorbed, indicating that most of the Ig produced in the course of the infection were not specific for the virus. Similar experiments with MHV, reovirus, and Sendai virus also showed that a large proportion of the IgG2a induced by the infection was not antiviral antibodies (data not shown). Screening of culture supernatants of hybridomas obtained with spleen cells from mice infected with adenovirus further confirmed that most B lymphocytes were induced to secrete IgG2a, whereas lymphocytes from uninfected mice were almost IgM producers (Table I). Moreover, only 1 of 56 supernatants of Ig-secreting hybridomas from infected animals produced antiviral antibody (data not shown), which strengthens the idea that the considerable increase of IgG2a observed after infection was not due to the production of antiviral antibodies and, therefore, that viruses can influence the switch of unrelated Ig.

The ability of viruses to influence IgG isotype was further established by infecting mice at the time of immunization with a soluble protein antigen. As shown in Table II, infection with some viruses dramatically modified the isotypic distribution of antibodies directed against tetanus toxoid, with a preferential production of IgG2a. This bias of the subclass distribution was especially striking when the virus induced a large increase of the antiprotein antibodies, as observed with MHV. Similar modifications of antiprotein isotypes were obtained with LDV, after immunization with transferrin and keyhole limpet hemocyanin (data not shown). By contrast, when mice were infected with a virus that did not induce any hypergammaglobulinemia, like polyomavirus, no modification in the antiprotein antibodies was observed.

As a whole, our results, as well as some isolated reports on LDV infection (4, 8, 9, and A. L. Notkins, unpublished observations), indicated that viral infections

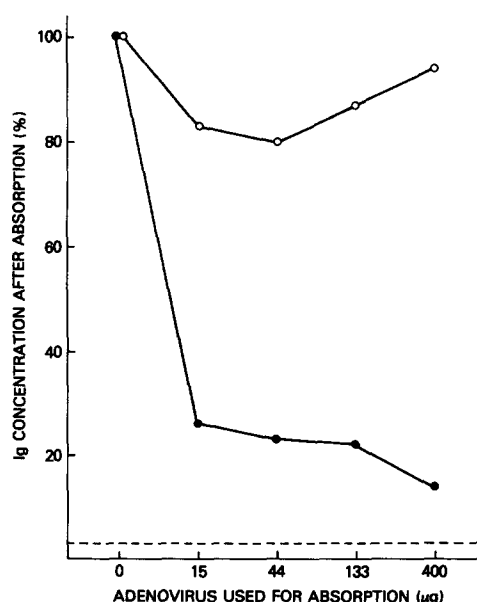


FIGURE 2. Absorption of antiadenovirus antibodies. Pooled serum from nine CBA/Rij mice infected for 3 wk with adenovirus was absorbed, as described in Materials and Methods, with serial doses of purified virus (6) or with buffer only. After removal of viral particles, remaining antiviral IgG antibody (●) and total IgG2a levels (○) were measured in supernatant by ELISA. Results are expressed in percent of the values obtained for serum absorbed with buffer only. The dotted line indicates the limit of detection for antiviral antibodies.

TABLE I
Spleen Cell Hybridization after Adenovirus Infection

Adenovirus infection	No. of wells tested	Proportion of wells positive for:				
		IgM	IgG1	IgG2a	IgG2b	IgG3
		%				
-	113	19	7	5	2	1
+	56	4	0	63	20	7

Spleen cells from groups of five control 129/Sv mice or of five animals infected for 2 wk with adenovirus were pooled and 10^8 cells were fused with SP2 *neo*^R at a 5:1 ratio (7). Ig isotypes were tested by RIA or ELISA in supernatants obtained from four and two independent hybridization experiments, for control and infected mice, respectively.

can influence the isotypic regulation of murine antibodies that are produced at the time of the infection. This observation strongly suggests that the isotypic bias in antiviral antibodies leading to a predominance of IgG2a (1) is not due to particular properties of viral antigens but to regulatory mechanisms that are triggered by viruses. So far, it is not known how viruses can modulate the IgG subclass distribution. Different reports (10-12) indicate that IFN- γ can stimulate the production of IgG2a from in vitro and in vivo activated B lymphocytes. As the T_H1 subset of T helper lymphocytes, which preferentially produce IFN- γ (13), has been shown to increase the secretion of IgG2a (14), it is tempting to speculate that the immune response triggered by viruses mainly involves this subset of cells. The antibody response after infection by viruses, and by some parasites (15), could therefore strongly differ from that elicited by immunization with protein antigens or by infection with some other parasites (16), which could rather involve the T_H2 subset of T lymphocytes that produce IL-4 (13), a lymphokine that stimulates the production of IgG1 and IgE antibodies (17, 18). However, it is quite possible that other mechanisms, like the release of soluble factors by infected cells, can also play a role in the selection of IgG2a antibodies during viral infections.

TABLE II
Effect of Viral Infection on Anti-tetanus Toxoid IgG Isotypes

Exp.	Virus	Anti-tetanus toxoid IgG				Total
		IgG1	IgG2a	IgG2b	IgG3	
		%				$\mu\text{g/ml}$
1	-	76.9 $\pm$ 5.4	19.9 $\pm$ 4.7	2.7 $\pm$ 0.2	0.5 $\pm$ 0.2	71.2 $\pm$ 17.3
	MHV	18.2 $\pm$ 7.7	76.7 $\pm$ 7.8	4.5 $\pm$ 0.7	0.5 $\pm$ 0.1	313.0 $\pm$ 97.8
	Adenovirus	37.1 $\pm$ 7.8	55.3 $\pm$ 7.2	6.8 $\pm$ 1.4	0.7 $\pm$ 0.1	67.5 $\pm$ 12.9
	Polyomavirus	76.3 $\pm$ 5.0	20.4 $\pm$ 4.5	2.9 $\pm$ 0.7	0.4 $\pm$ 0.1	58.2 $\pm$ 7.7
2	-	82.2 $\pm$ 4.1	12.0 $\pm$ 2.1	5.6 $\pm$ 2.4	0.2 $\pm$ 0.03	73.8 $\pm$ 4.3
	LDV	46.1 $\pm$ 3.0	48.2 $\pm$ 2.9	5.2 $\pm$ 0.6	0.5 $\pm$ 0.1	99.5 $\pm$ 18.9

Groups of three to six 129/Sv mice were infected 1 d before immunization with 100 μg tetanus toxoid. Anti-tetanus toxoid IgG subclasses were measured by ELISA in individual sera obtained 3 wk later. Results are expressed in percent (mean $\pm$ SE) of the total anti-tetanus toxoid IgG (sum of the four subclasses).

Because IgG2a antibodies have functional properties different from those of other isotypes, which could be necessary in the defense against viruses (19–21), the preferential production of this subclass of Ig could correspond to a more appropriate response of the infected organism to the virus. This could be of importance for the development of vaccination schemes, especially since preliminary experiments (data not shown), as well as a recent study on influenza virus (22), suggest that immunization with inactivated virus or with viral protein may elicit IgG responses with a subclass distribution similar to that of antiprotein antibodies. On the other hand, the ability of viruses to modify the isotype of irrelevant antibodies could potentially play a role in the pathogenesis of autoimmune diseases by increasing the deleterious effect of autoantibodies produced during the course of an infection.

Summary

The isotypic distribution of murine IgG was examined after infection with several viruses. The results indicate that when a hypergammaglobulinemia was induced by the infection, it was restricted to the IgG2a and, to a lesser extent, to the IgG2b subclasses. In addition, when mice were infected with some viruses concomitantly with the immunization with a soluble protein antigen, a modification in the isotypic distribution of antiprotein antibodies was observed, with a preferential production of IgG2a. These observations indicate that viral infections can actively influence the switch of Igs and selectively stimulate the production of the IgG2a subclass.

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