

IgG2a RESTRICTION OF MURINE ANTIBODIES ELICITED BY VIRAL INFECTIONS

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Murine antibody responses to soluble proteins and to carbohydrates are generally restricted to the IgG1 and IgG3 subclasses, respectively (1–7), suggesting that IgG isotypes are not selected at random. Surprisingly, no family of antigens has been shown to preferentially induce the production of IgG2a antibodies, although this subclass represents a major component of mouse serum immunoglobulins (8). A few recent observations nevertheless suggested that IgG2a could predominate in antiviral antibody responses (9–12). This isotypic bias was particularly striking after infection with lactate dehydrogenase-elevating virus (LDV)¹ and lymphocytic choriomeningitis virus (LCMV). However, because these two viruses induce considerable alterations of the immune system, including a polyclonal B lymphocyte activation (13, 14), they may evoke antibody responses that are not representative of more common antiviral reactions.

To examine whether IgG2a restriction is a general property of murine antibody responses to viral antigens, we analyzed the isotypic profile of antibodies directed against a panel of both DNA and RNA mouse viruses, representative of widely different genera. Our data demonstrate that all viral infections introduce a unique bias in the subclass selection process that makes IgG2a the predominant antiviral IgG antibody in the mouse.

Materials and Methods

Mice. CBA/Rij and 129/Sv mice were bred at The Ludwig Institute for Cancer Research, and C57BL/6 mice were purchased from TNO (REPGO Institute TNO, Rijswijk, The Netherlands). Mice were maintained in specific pathogen-free conditions and used when 6–10 weeks old.

Viruses. Infections were performed by the intranasal route with serial doses of a panel of viruses, including mouse adenovirus (FL strain), polyomavirus (LID-1 strain), reovirus type 3 (Abney strain), sindbis virus (Ar-339 strain), mouse hepatitis virus (MHV; A59

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¹ *Abbreviations used in this paper:* LDV, lactate dehydrogenase-elevating virus; LCMV, lymphocytic choriomeningitis virus; MHV, mouse hepatitis virus; PVM, pneumonia virus of mice; TMEV, Theiler's mouse encephalomyelitis virus.

strain), Sendai virus, pneumonia virus of mice (PVM; strain 15), LCMV (WE3 strain), Theiler's mouse encephalomyelitis virus (TMEV; GDVII strain), and Mengo virus (C50L strain), or by the intraperitoneal route with LDV (Riley strain).

Antigens. Mice were immunized by intraperitoneal injection without adjuvant of 100 μ g of chicken immunoglobulin (a gift of Dr. J. P. Vaerman, Université Catholique de Louvain), human lactoferrin (15), horse ferritin (Boehringer Mannheim, Mannheim, Federal Republic of Germany), bovine fibrinogen (Calbiochem-Behring Corp., La Jolla, CA), tetanus toxoid (Wellcome Reagents Limited, London, England), human thyroglobulin (a gift of Dr. P. De Nayer, Université Catholique de Louvain), bromelain (Calbiochem-Behring Corp.), human IgE (a gift of Dr. J. M. Saint-Remy, Université Catholique de Louvain), keyhole limpet hemocyanin (KLH) (Calbiochem-Behring Corp.), or human transferrin (Behringwerke, AG, Marburg/Lahn, Federal Republic of Germany).

ELISA. IgG antibody subclasses were determined in sera by ELISA as described by Coutelier et al. (10). Briefly, polystyrene plates (439454; Nunc, Roskilde, Denmark; or 655101; Greiner, Nürtingen, Federal Republic of Germany) were coated overnight with pelleted viruses or with purified proteins, and incubated with serial dilutions of sera. Binding of antibodies was measured with rabbit antisera specific for each mouse IgG subclass followed by peroxidase-conjugated goat anti-rabbit IgG antibodies, or, for C57BL/6, IgG2a, with an allotype-specific monoclonal antibody of BALB/c origin. Results, expressed in micrograms per milliliter, were calculated on standard curves of selected anti-DNP monoclonal antibodies (10).

Results and Discussion

The subclass distribution of serum IgG antibodies was analyzed after infection of mice with a panel of both DNA and RNA viruses, representative of eleven different genera. For comparison, animals were also immunized with a number of soluble protein antigens. These two groups of antigens elicited specific antibody responses that differed markedly in their isotypic profiles. As a rule, viruses induced much more IgG2a than IgG1 antibodies, while the converse was observed with soluble proteins. This was best illustrated by the IgG2a/IgG1 ratio shown in Fig. 1. Somewhat surprisingly in view of the heterogeneity of the viruses used in our experiments, the predominance of IgG2a antibodies was consistent, ranging from 65 to 92% of total antiviral IgG in CBA/Rij mice (Table I). In particular, it was not significantly affected either by the presence or absence of a viral envelope or by the DNA or RNA nature of the viral nucleic acids. Moreover, it remained relatively constant with time (data not shown) and was not changed after a secondary infection (Table II). The possibility that this restriction could be due to the intranasal route used for virus inoculation was also excluded by the finding that intraperitoneal injection of adenovirus elicited a similar IgG2a-restricted response (Fig. 2).

In contrast, IgG1, IgG2b, and IgG3 antibodies showed greater relative variations. For example, virtually no IgG1 antibodies were detectable against polyomavirus or sindbis virus, whereas they represented ~20% of the antibody response against Sendai virus. These differences were reproducible from one experiment to another, which suggests that each viral species elicits an antibody response with a characteristic isotypic profile.

The isotypic pattern of antiviral antibodies varied little in the three strains of mice tested (129/Sv, CBA/Rij, and C57BL/6). However, after infection with certain viruses, such as TMEV and PVM, C57BL/6 produced much less IgG2a than CBA/Rij and 129/Sv mice (Table III). This observation, which fits well

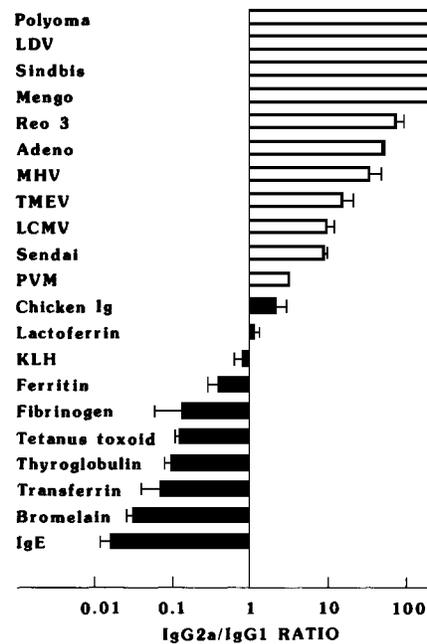


FIGURE 1. IgG2a/IgG1 ratio of antiviral and antiprotein antibodies. Sera from 129/Sv mice were collected 6–7 wk after infection or 3–6 wk after immunization with soluble proteins. Results are expressed as log mean \pm SE of individual IgG2a/IgG1 ratios for antiviral (open bars) and antiprotein (closed bars) antibodies. The broken line indicates the minimal IgG2a/IgG1 ratio for mice where no IgG1 could be measured.

TABLE I
Isotypic Distribution of Antiviral IgG Antibodies in CBA/Rij Mice

Virus	IgG1	IgG2a	IgG2b	IgG3
Adeno	2.3 \pm 0.3	91.6 \pm 0.6	4.4 \pm 0.4	1.6 \pm 0.2
Polyoma	<0.2	83.0 \pm 2.2	9.9 \pm 1.9	7.1 \pm 1.4
Reo 3	<0.3	86.8 \pm 1.2	12.5 \pm 1.2	<0.4
Sindbis	0.8 \pm 0.1	90.3 \pm 1.0	7.7 \pm 0.8	1.3 \pm 0.2
LDV	3.0 \pm 1.1	74.6 \pm 3.3	13.0 \pm 2.3	9.4 \pm 2.1
MHV	7.9 \pm 2.8	79.0 \pm 1.6	10.4 \pm 1.1	2.7 \pm 0.5
Sendai	17.6 \pm 4.8	75.3 \pm 6.3	6.7 \pm 1.5	0.4 \pm 0.1
PVM	13.0 \pm 3.0	66.1 \pm 4.4	15.6 \pm 1.9	5.3 \pm 2.5
LCMV	10.1 \pm 1.7	86.6 \pm 1.8	3.1 \pm 0.3	0.4 \pm 0.1
TMEV	12.4 \pm 2.2	65.9 \pm 3.6	19.4 \pm 3.6	2.4 \pm 0.3
Mengo	<5.2	71.2 \pm 5.1	18.8 \pm 3.9	<4.8

Results are expressed in percent total antiviral IgG (sum of the four subclasses), mean \pm SE. 7–17 mice/group were tested 21 d after infection.

with previous analyses of anti-LDV (10) and antiioxazolone (16) antibodies, could be related to the Igh-C allotype of the mice.

The unusual isotypic profile of antiviral antibodies could either be due to certain common biochemical properties of viral antigens, such as the presence of repetitive determinants, or be related to the viral infection itself. Preliminary results indicated that, in some cases, a concomitant infection could modify the

TABLE II
Isotypic Profile of Anti-TMEV Antibodies after Secondary Infection

Time after secondary infection*	Total IgG	IgG1	IgG2a	IgG2b	IgG3
<i>d</i>	$\mu\text{g/ml}$				% [‡]
-1	1.9 ± 0.3	23.7 ± 5.4	68.1 ± 6.2	6.9 ± 0.8	1.3 ± 0.3
8	1.7 ± 0.3	13.9 ± 2.5	73.7 ± 2.7	11.5 ± 1.2	1.0 ± 0.2
49	3.5 ± 0.2	11.1 ± 0.6	84.0 ± 1.0	4.5 ± 1.6	0.4 ± 0.2

* CBA/Rij mice (3–4 mice/group) were reinfected with TMEV 7 wk after a primary infection.

[‡] Results are expressed as percent total antiviral IgG (mean \pm SE).

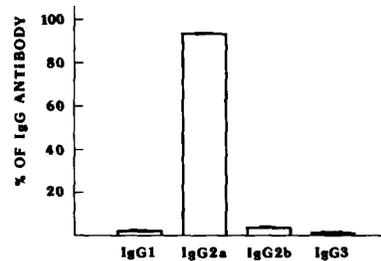


FIGURE 2. Isotypic distribution of antiadenovirus antibodies after intraperitoneal infection. Sera were collected 3 wk after intraperitoneal infection of 129/Sv mice. Results are expressed as percent total IgG response, (mean \pm SE, $n = 20$).

TABLE III
Isotypic Distribution of Antiviral IgG Antibodies in C57BL/6 Mice

Virus	IgG1	IgG2a	IgG2b	IgG3
Adeno	0.3 ± 0.1	85.4 ± 0.7	11.8 ± 0.7	2.5 ± 0.2
Sendaï	11.1 ± 1.4	73.4 ± 2.2	14.7 ± 1.1	0.8 ± 0.1
PVM	33.9 ± 3.8	33.2 ± 3.4	19.5 ± 2.8	13.5 ± 2.7
LCMV	2.8 ± 0.7	81.8 ± 1.8	12.9 ± 0.9	2.6 ± 1.0
TMEV	6.5 ± 2.4	57.6 ± 4.8	34.7 ± 3.1	1.3 ± 0.1
Mengo	<0.3	90.3 ± 1.8	9.0 ± 1.8	0.4 ± 0.1

Results are expressed as percent total antiviral IgG (sum of the four subclasses), mean \pm SE. 9–18 mice/group were tested 21 d after infection.

isotypic distribution of the antibody response against inactivated virus, which supports the latter hypothesis. Analysis of the mechanisms involved in this phenomenon could provide a new approach to the study of isotype selection.

IgG2a antibodies have been shown (17–19) to display functional characteristics different from IgG1 immunoglobulins, in particular with regard to complement activation. The predominance of this subclass after viral infection could therefore correspond to the selection of a more efficacious response to the virus. If this proved to be true, this observation would have major implications in the development of appropriate vaccination schemes.

Summary

The isotypic distribution of IgG antibodies was determined in the serum of mice after infection with a panel of RNA and DNA viruses representative of 11

different genera. The antiviral response induced by all these viruses showed a striking preponderance of the IgG2a subclass whatever the strain of mice tested or the time elapsed after infection. Together with the predominance of IgG1 in antiprotein and of IgG3 in anticarbohydrate responses, this IgG2a restriction of antiviral antibodies strongly suggests the existence of highly specific mechanisms for the regulation of individual subclasses in the mouse.

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