RESPIRATORY VIRUS-INDUCED IMMUNOSUPPRESSION IN MICE

E. V. Sidorova, M. G. Agadzhanyan, L. A. Mazhul',
I. Beladi, M. Bakay, and K. Berencsi

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Changes in function of the immune system are observed in many virus infections. Many viruses lead to its suppression and thereby weaken the resistance of the host accompanying infections. The list of viruses with immunomodulating properties includes, in particular, such widely spread respiratory viruses as adenoviruses and influenza viruses [2, 5, 6]. The study of the mechanisms of the immunosuppressive action of these viruses is clearly important for the development of methods of immunocorrection in viral infections.

Inhibition of the immune response of mice to a heterologous antigen, namely sheep's red blood cells (SRBC), by human type 6 adenovirus (Ad6) was demonstrated previously [5, 6]. It was decided to study whether influenza viruses of strains A/PR/8/34 (A/PR/8) and A/Krasnodar (A/Kr) possess a similar nonspecific inhibitory action. It was also necessary to establish whether the effects observed are linked only with suppression of the response to the heterologous antigen used, or whether the suppression is polyclonal in character, and that adenovirus, for example, can depress the immune response to influenza virus, and various strains of influenza viruses can modulate responses of one to another. The aim of the investigation described below was to shed light on these questions.

EXPERIMENTAL METHOD

Female BALB/c mice weighing 14-16 g were obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR.

Native and UV-inactivated strains of viruses A/PR/8, A/Kr, and Ad6, and also noninfectious antigens (SRBC) and polyvinylpyrrolidone with mol. wt. of 350 kD (PVP_{350}) were used as antigens.

The animals were immunized by intravenous or intraperitoneal injection of influenza viruses (10-15 μg per mouse as protein) or 10^{11} $TCID_{50}$ of Ad6, and heterologous antigens were injected at different times thereafter (from a different strain of virus, PVP_{350}, or SRBC). Intact mice and mice receiving only the test antigens at the same times as the experimental mice served as the control. On the 4th day after injection of the test antigens the number of antibody-forming cells (AFC) to SRBC [7], PVP_{350} [8], and influenza viruses [3] and also the number of immunoglobulin-forming cells (IGFC) [9] in the spleens of individual mice were determined. SRBC sensitized with affinity-purified antibodies to mouse immunoglobulin [9] were used as indicator erythrocytes in the last case, and rabbit antiserum against mouse immunoglobulins in a dilution of 1:100 was used as the intensifying serum (it was shown previously that with this dilution the largest number of IGFC of all isologous types could be detected). The results were expressed as arithmetic mean values of the number of AFC and IGFC per 10^6 splenocytes (M) ± the arithmetic mean error (m).

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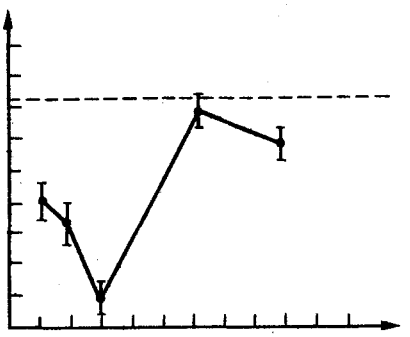

Fig. 1. Dependence of immune response to heterologous antigen (SRBC) on time between injection of influenza virus (A/PR/8) and this antigen Abscissa: —5, —4, —3, —2, —1, 0, +1, +2, +3, +4, +5. Time interval between injection of A/PR/8 and SRBC. Minus sign indicates virus injected before SRBC, zero indicates virus and SRBC were injected simultaneously, plus sign virus injected after SRBC. Ordinate: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10. Number of AFC to SRBC per 10^6 splenocytes ($\cdot 10^{-2}$), determined 4 days after immunization with SRBC. Broken horizontal line shows number of AFC to SRBC in mice receiving antigen alone (control — 733 ± 97).

EXPERIMENTAL RESULTS

The experiments with type 6 adenovirus (Ad6) showed that its immunosuppressive action is exhibited only under the following conditions: in response to injection of living virus, with a certain time interval between injection of the virus and antigen, and with injection of Ad6 and heterologous antigen (SRBC) by the same route, preferably intraperitoneally [5, 6]. It was necessary to determine whether the same rules still apply when influenza viruses were used as immunomodulators.

The first step was to study the dynamics of formation of AFC to SRBC after intraperitoneal injection of SRBC at various time intervals after intraperitoneal immunization of the mice with virus A/PR/8 (Fig. 1).

In normal mice, just as in animals receiving the viruses alone, the number of AFC to SRBC did not exceed 2 or 3 per 10^6 splenocytes (data not shown). Injection of A/PR/8 simultaneously with SRBC did not affect the response to SRBC (724 AFC per 10^6 splenocytes compared with 733 AFC in the control). By contrast, injection of the virus 5-3 days before injection of SRBC led to appreciable inhibition of the response to SRBC, which was most marked when the virus was given 3 days before the antigen (Fig. 1): the number of AFC to SRBC fell from 733 per 10^6 splenocytes (in mice receiving the antigen alone) to 99 per 10^6 splenocytes (in mice receiving influenza virus A/PR/8 before SRBC). Thus maximal immunosuppression in this series of experiments amounted to about 86%.

According to the results of 21 experiments the mean suppression of the specific response to SRBC under these experimental circumstances was 69 ± 16% (Table 1).

Just as with Ad6 [5, 6], injection of virus A/PR/8 and of SRBC by different routes caused virtually no suppression of the immune response. For instance, when A/PR/8 was injected intravenously into mice and SRBC was injected intraperitoneally 3 days later, the immune response to antigen in the experimental group was not less than 92% of that in the control. Similar results were obtained also when the experiment was performed the other way round (intraperitoneal injection of virus intravenous injection of antigens).

It will also be noted that when A/PR/8 virus inactivated by UV irradiation was used, suppression of the immune response to SRBC was not observed (data not shown) although, as was shown previously [1], the immunogenicity of the viral preparation was completely preserved. This indicates that virus-induced immunosuppression is not simply the result of antigenic competition, but is also caused by certain other as yet unexplained mechanisms. It is accordingly interesting to

TABLE 1. Immunosuppression Induced by Influenza and Ad6 Viruses

Virus administered	Antigens used for immunizing mice				Number of AFC per 10^6 mouse splenocytes to antigens				Number of IGFC per 10^6 cells
	SRBC	PVP_{350}	A/PR/8	A/Kr	SRBC	PVP_{350}	A/PR/8	A/Kr	
—	+	—	—	—	853±165 (*n*=28)	—	—	—	2394±543 (*n*=15)
A/PR/8	+	—	—	—	240±149 (*n*=21)	—	—	—	970±235 (*n*=12)
—	—	+	—	—	—	64±24 (*n*=9)	—	—	2272±337 (*n*=6)
A/PR/8	—	+	—	—	—	55±27 (*n*=11)	—	—	1173±614 (*n*=2)
—	—	—	—	+	—	—	—	106±10 (*n*=5)	2353±545 (*n*=2)
A/PR/8	—	—	—	+	—	—	—	32±10 (*n*=6)	2042±214 (*n*=3)
—	+	—	—	—	930±150 (*n*=9)	—	—	—	—
A/Kp	+	—	—	—	607±108 (*n*=6)	—	—	—	—
—	+	—	—	—	1914±251 (*n*=3)	—	—	—	11 309±1182 (*n*=3)
Ad6	+	—	—	—	184±36 (*n*=5)	—	—	—	2175±289 (*n*=5)
—	—	—	Inf +	—	—	—	1128±371 (*n*=9)	—	6813±447 (*n*=9)
Ad6	—	—	Inf +	—	—	—	280±98 (*n*=8)	—	2972±603 (*n*=8)
—	—	—	+ "killed"	—	—	—	1574±189 (*n*=9)	—	6608±580 (*n*=9)
Ad6	—	—	+ "killed"	—	—	—	389±55 (*n*=10)	—	2997±217 (*n*=10)

Legend. Viruses A/PR/8 and A/Kr injected 3 days before heterologous antigens, Ad6 5 days before. n) Number of mice in experiment. All injections given intraperitoneally. Inf) Infectious.

note that in experiments in which the nonpathogenic A/Kr strain was used instead of the pathogenic A/PR/8 strain of the virus the level of immunosuppression of the specific response to SRBC was considerably lower (Table 1),

The action of A/PR/8 on IGFC formation repeated the picture of suppression of AFC formation, although it was rather weaker (Table 1). For instance, if the virus was injected 3 days before SRBC, i.e., at the peak of suppression of the specific response, the mean number of IGFC was 970 ± 235 compared with 2394 ± 543 per 10^6 cells in the control (mice receiving SRBC only), Inhibition of the response was thus about 59.0.

In the experiments described above a T-dependent noninfectious antigen (SRBC) was used as the heterologous antigen. It was interesting to discover whether similar suppression takes place in relation to the immune response to T-independent antigen, and whether one species of virus can inhibit the response to another species (or strain) of virus, for when living antigens are used, relations between them are inevitably more complex than when noninfectious antigens are used. Accordingly, we carried out experiments to determine the immunomodulating action of A/PR/8 on the immune response to T-independent antigen PVP_{350}, and also experiments to determine the effect of A/PR/8 on the immune response to A/Kr and the effect of Ad6 on the immune response to living and UV-killed A/PR/8.

The data in Table 1 show that the action of A/PR/8 on the immune response to T-independent antigen (PVP_{350}) does in fact differ from its action on the response to T-dependent antigen. Whereas the response to SRBC was inhibited by almost 70% in the case of preliminary injection of the virus, the specific response to P_{350} was virtually not reduced. This suggests involvement of T cells in A/PR/8 induced immunosuppression.

The data in Table 1 also are evidence that the response to T-dependent antigens is inhibited irrespective of whether these antigens were used as infectious or noninfectious agents. For instance, preliminary injection of Ad6 5 days before A/PR/8 inhibited the immune response to influenza virus by 76%. It was immaterial whether the A/PR/8 virus used was infectious or inactivated by UV irradiation (suppression of the response was virtually equal).

Thus injection of a virus of one species can inhibit the immune response to virus of another species. Moreover, even different strains of the same virus can evidently suppress the specific immune response to one another. Thus the preliminary injection of A/PR/8 3 days before injection of A/Kr depressed the specific response to the latter by about 30%.

The results indicate that the immunosuppressive action of viruses is polyclonal in character, and that T lymphocytes can play a definite role in this process.

At the same time, PVP_{350} is known to be a type 2 T-independent antigen, and during the immune response to such antigens, just as to T-dependent antigens, suppressor T cells are formed [4, 8]. This suggests that not suppressor T cells, but other subpopulations of T cells participate in virus-induced immunosuppression. It is not clear, however, why the most effective method of inducing immunosuppression is the intraperitoneal injection of infectious virus, and why immunosuppression is observed only when viruses and antigens are injected by the same route. Previous data showed the possible involvement of peritoneal macrophages in Ad6-induced immunosuppression [5]. However, no direct proof of the role of macrophages, nor indeed to T-lymphocytes, in this process has yet been obtained.

The problems raised by this investigation can be solved by the creation of in vitro model systems of immunosuppression and the study of the role of individual cell populations in them.

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