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The specificity of the antibody responses of human volunteers to certain respiratory viruses

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Minor upper respiratory infections are due to a great many viruses, belonging to several biological groups, and one of the most important of these is the picornavirus group (Report, 1963). This includes, on the one hand, the enteroviruses, polioviruses, echoviruses and coxsackieviruses, and on the other, the rhinoviruses which are classified as M rhinoviruses or H rhinoviruses according to whether they can grow in both monkey and human cells (M) or in human cells only (H).* Enteroviruses often invade the upper respiratory tract and may cause illness with symptoms such as sore throat in addition to constitutional upset. Rhinoviruses cause common colds (Tyrrell & Chanock, 1963). It seems probable that the average man is infected with a succession of enteroviruses of different serotypes, particularly in childhood, and with a similar succession of rhinoviruses, in childhood, adolescence and in later life. Following infection, antibodies against the invading virus are usually produced. Generally speaking the possession of neutralizing antibody is correlated with resistance to infection by the corresponding enterovirus or rhinovirus.

Almost all picornaviruses seem to be antigenically quite distinct from each other when studied by cross-neutralization tests with sera from experimentally inoculated animals, but when studied by complement fixation (CF) cross-reactions between enteroviruses may be detected. When paired sera from subjects who are naturally infected with enteroviruses are tested by CF, antibody responses to several viruses may be found; these are less commonly observed when sera are tested by neutralization.

Mogabgab (1962*a*) has recently stressed the fact that, using neutralization and haemagglutination-inhibition as well as complement-fixation tests, he detected in the sera of patients suffering from acute respiratory infections simultaneous antibody rises against a number of enteroviruses and rhinoviruses. He draws the conclusion that the viruses are so interrelated that a specific serological diagnosis is impossible. He also believes that vaccination with one or two viruses of this group might protect against a large number of the viruses which cause minor upper respiratory infections.

* The rhinoviruses have not yet been allocated to numbered serotypes as have the enteroviruses and so they are referred to by initials or laboratory numbers. In addition, one rhinovirus was originally designated as ECHO 28, and the name will be printed thus, with capital letters, in order to distinguish between it and typical echoviruses printed with small letters in accordance with the new convention.

This paper reports the antibody responses of volunteers inoculated with picornaviruses which are known to infect the respiratory tract. Sera kept from experiments performed during the past few years have been tested against a variety of viruses; the results bear on the specificity of antibody rises, the mechanism by which immunity is built up in natural conditions and the likelihood of enhancing general immunity by means of a vaccine containing a few picornaviruses.

MATERIALS AND METHODS

All volunteers were aged between 18 and 50 years and most were kept in isolation. One group was inoculated intranasally with echovirus 11 (strain U) passed in tissue culture (Buckland, Bynoe, Philipson & Tyrrell, 1959) and another with a strain of coxsackievirus A 21 which had been passed in tissue cultures of human amnion cells (see Parsons, Bynoe, Pereira & Tyrrell, 1960), back to man and then once more into tissue culture, this time of human embryo kidney. Other volunteers were inoculated with an H rhinovirus, D.C., which had been passed in tissue cultures and again in man (Tyrrell, Bynoe, Buckland & Hayflick, 1962). The pairs of sera studied all showed an antibody rise against the infecting virus. Other volunteers received active M rhinovirus, P.K., passed 8 times in human embryo tissue cultures and administered as a single intramuscular injection of 1 ml. of undiluted tissue culture fluid. Still others were given a formalin-inactivated M rhinovirus, ECHO 28, as another experimental vaccine, but this last group of volunteers was not kept in isolation. These intramuscular injections produced particularly high antibody responses against the homologous virus (Doggett, Bynoe & Tyrrell, 1963) and therefore these sera were chosen in preference to those of volunteers who were given virus by the intranasal route.

The sera were collected between 2 and 3 weeks after the administration of virus and had all been kept at $-20°$ C. after being collected; although rather small volumes remained of some of these it was possible to test them by using (1) the micromethod of Takátsy & Furesz (1954) as modified by Sever (1962) for the haemagglutination-inhibition tests (HI) and (2) the microplaque reduction test (Taylor-Robinson & Tyrrell, 1962*a*) for measuring neutralizing activity (K). Previous experiments had shown that very similar results were obtained with the HI test and neutralization tests using either echovirus 11 or coxsackievirus A 21, so the HI test was used because it was simple and economical.

RESULTS

Heterotypic antibody responses were rarely found. Their distribution can be seen from Table 1. The detailed results in typical cases in which no heterotypic rise occurred and in those in which it did are shown in Table 2. Earlier studies had shown that antibody rises against M rhinovirus, B 632, occurred in volunteers vaccinated with ECHO 28 virus (Doggett *et al.* 1963; Mogabgab, 1962*b*). The further experiments reported here show that heterotypic responses did not occur against picornaviruses in general. The rise in antibody against the serologically distinct M rhinovirus H.G.P. occurred in a volunteer who was inoculated with ECHO 28 virus before coming to the Unit and who was therefore not isolated at

Table 1. *Summary of antibody responses to two enteroviruses and three rhinoviruses*

Virus strain given	Type	Administration	Geometric mean rise against virus given	4-fold or greater antibody* rises against			
				Coe	U	H.G.P.	ECHO 28
Coe	Coxsackievirus A21	Nasal drops	16·3	6/6†	1/6	0/5	0/5
U	Echovirus 11	Nasal drops	13·1	1/9	9/9	0/9	0/9
D.C.	H Rhinovirus	Nasal drops	8·1	0/7	0/7	0/7	0/7
P.K.	M Rhinovirus	Intramuscular (isolated)	17·2	0/6	0/6	6/6	0/6
ECHO 28	M Rhinovirus	Intramuscular (not isolated)	6·8	0/5	0/5	1/5	5/5

* Antibody against Coe and ECHO 11 was measured by HI and against the rhinoviruses by neutralization. Only the sera from volunteers given D.C. were titrated with D.C.

† Numerator = number showing rise. Denominator = number tested. P.K. and H.G.P. are antigenically identical.

Table 2. *The usual, specific antibody responses and three unusual heterotypic responses*

Virus strain given	Volunteer	Antibody titre against			
		U*	Coe*	H.G.P.†	ECHO 28†
U	Tu.	<4/32	<4/<4	0·21/0·35	0·23/0·23
U	Ja.	<4/32	<4/8	2·5/2·5	2·7/1·9
Coe	Sm.	<4/<4	16/256	1·5/2·4	>54/35
Coe	Ga.	<4/8	4/256	—	—
ECHO 28	Jo.	<4/<4	<4/<4	5·7/6·1	0·41/4·9
ECHO 28	Ho.	<4/<4	4/<4	0·3/>7·4	<0·1/1·2

* HI antibody titre—numerator = titre of first specimen and denominator = titre of second specimen.

† Serum neutralizing activity (K value).

the time; he developed a cold between the time of inoculation and the collection of serum after vaccination. It was not possible to test his nasal secretions for the presence of a virus, but he might have been infected with an agent antigenically related to H.G.P. Additional experiments were done on the sera of two volunteers who had been infected by intranasal inoculation with B632 and two with ECHO 28. These showed that although there was an antibody rise against ECHO 28 in one subject infected with B632, and a rise against B632 in one subject infected with ECHO 28, there was no rise in antibody against Coe or H.G.P.

Inspection of the original data shows that there were no regular small rises in antibody titre against heterotypic viruses which might have been significant in total although too small individually to be so regarded. In fact, small falls were about as frequent as small rises, and probably both reflected random errors as a result of the method of titration used.

All in all, heterotypic antibody rises were found in three tests whereas they might have been found in 104 if heterotypic responses had occurred generally.

DISCUSSION

In earlier experiments it was shown that when adults were injected intramuscularly with ECHO 28 virus there were frequent heterotypic responses to M rhinoviruses strain B632 (Doggett *et al.* 1963) or K779 (Mogabgab, 1962*b*); the latter two strains are very similar antigenically (Tyrrell unpublished) and can be shown to be related to ECHO 28 by neutralization tests on sera prepared in rabbits (Taylor-Robinson & Tyrrell, 1962*b*). In our present studies we can find little evidence that infection or vaccination with one serotype of the viruses used in this study induced antibody responses against the others to any important degree although there appears to be a slight cross-reaction between coxsackievirus A21 and echovirus 11. Ketler, Hamparian & Hilleman (1962) measured neutralizing antibody rises in patients infected naturally with antigenically distinct strains of rhinovirus; the responses seemed to be completely specific, but this may have been partly because only low homologous titres were detected.

Mogabgab showed a high frequency of heterotypic antibody responses; this may have been (1) because his subjects were not isolated, and therefore could have had double infections, (2) because they had previous experience of a wider variety of picornavirus than our volunteers, (3) because the infecting viruses stimulated more antibodies than did ours, or (4) because the viruses used in his serological tests were different; in this connexion he himself believed that the strains he used, which had been passed in KB cells, had been made thereby better detectors of heterotypic antibody (Mogabgab, 1962*a*). Possibility (1) above suggests that our results may be more significant than Mogabgab's. Although (2) cannot be tested we think that the groups are likely to be of similar socioeconomic background and therefore to have had similar experiences. Regarding (3) the illnesses he studied may have been more severe than ours, but the antigenic stimulus of an intramuscular injection of H.G.P. and ECHO 28 appears to be greater than that of a nasal infection. Regarding (4) we know that antibody measurements of the type we made can be well correlated with the immunity of the subject (Bynoe, Hobson, Horner, Kipps, Schild & Tyrrell, 1961; Tyrrell, 1963). We therefore feel that although the technique used by Mogabgab might have broadened the activity of the antigen in a way which had some advantages for a diagnostic test, our results can be more readily interpreted in terms of the probable changes in the immunity of the subjects.

In view of all the evidence we believe that antibodies to the viruses studied are usually acquired one-by-one following infection with each new serotype. In addition we would not expect a broad immunity to follow vaccination with any one of the strains used in these studies.

SUMMARY

Experiments reported here were performed on the sera of thirty-three volunteers who were infected or inoculated intranasally or intramuscularly in groups of five to nine with one of two enteroviruses, namely, echovirus 11 and coxsackievirus A21 or with one of three rhinoviruses, namely, M viruses P.K. and ECHO

28, and H virus D.C. Antibody responses occurred against the virus administered, and rarely against the other viruses used. It was concluded that although these viruses are related biologically the antibody responses in the volunteers were largely specific.

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