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The use of monoclonal antibodies for the antigenic analysis of influenza A viruses

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Monoclonal antibodies have been found to provide useful additional information for the antigenic analysis of influenza A viruses of the H3N2 and H1N1 subtypes. They have been particularly useful in the interpandemic period when multiple variants circulate concurrently.

Apparently heterogeneous isolates can be placed in fairly clear-cut groups on the basis of their reactivity with certain monoclonal antibody preparations. It is thought likely that variants reacting with the least number of monoclones are the most different antigenically from the fully reactive strains.

When a new subtype of influenza A virus appears, the viruses isolated in the first epidemics are usually antigenically identical, showing no difference in reactivity in haemagglutination-inhibition (HI) tests with specific ferret antisera. Differences in avidity caused problems in the identification of early H2N2 viruses (*1*) but were not a feature of the later H3N2 subtypes, and the identity of isolated viruses has usually been confirmed quickly and accurately with a small battery of specific antisera.

However, once the first epidemics are over, variants begin to appear, even as early as the second season in the case of the H3N2 subtype (*2*). These variants are recognized by differences in their reactions in HI tests, and these antigenic modifications have been shown to be associated with changes in the amino acid sequence of the haemagglutinin (*3*).

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In the early years of influenza epidemiology, it was thought that, when an antigenic variant appeared, it would replace the previously circulating strains and itself become predominant. The detection of such variants, together with epidemiological evidence such as the occurrence of significant outbreaks, has therefore formed the basis for decisions on which viruses should be incorporated in vaccines for use in the following season.

For several years now, it has been clear that this simplistic interpretation is no longer tenable and that several variants may circulate concurrently (*4*), each with a different antigenic specificity and divergence from the earlier viruses of the same subtype. It is often difficult to know which of these several variants should be selected for inclusion in a vaccine.

Such a situation has arisen in recent years when two different subtypes, H3N2 and H1N1, have been circulating, both undergoing rather slow and irregular antigenic changes.

Ferret antibodies, as postinfection convalescent antisera, can be produced rapidly, are specific, and

Table 1. Cross-reactivity of H1N1 prototypes with ferret antisera and monoclones

	Postinfection ferret serum				Monoclones to A/USSR/90/77				No. of isolates in United Kingdom				
Virus	A/USSR/ 90/77	A/Brazil/ 11/78	A/England/ 333/80	A/Texas/ 29/82	264/2	110/1	18/3	22/1	1977-78	1978-79	1979-80	1980-81	1981-82
A/USSR/90/77	640	160	320	< 40	+ +	+ +	+ +	+ +	31	17	0	0	0
A/Brazil/11/78	160	320	160	< 40	–	+ +	+ +	+ +	12	4	4	0	0
A/England/333/80	320	320	1280	160	–	–	+ +	+ +	0	8	8	177	2
A/Texas/29/82	< 40	< 40	40	1280	–	–	–	+ +	0	0	0	0	2

remain indispensable for the preliminary identification of influenza viruses. In a period of slow drift, however, the differences in reactivity may not be sharp enough to allow an antigenic analysis. Monoclonal antibodies have therefore been introduced into the identification procedures, to attempt to obtain extra information that would show more clearly what antigenic changes were occurring among the influenza viruses circulating in the world.

This paper describes the results obtained using monoclonal antibodies with antigenic variants of both the H3N2 and H1N1 subtypes of influenza A viruses isolated in recent years.

MATERIALS AND METHODS

Influenza viruses

Viruses isolated in laboratories in several countries were received as either tissue cultures (simian kidney, LLCMK2, or MDCK) or allantoic fluids. These materials were tested directly for the presence of haemagglutinin with both chicken and guinea-pig erythrocytes; if the titre was high enough, HI tests were done with a carefully measured dose of haemagglutinin against a battery of ferret antisera. If insufficient haemagglutinin was detected, the virus was inoculated into the allantoic cavity of 10-day fertile hens' eggs and HI tests were done with the infected allantoic fluid after 48 hours' incubation.

Ferret antisera

Ferret antisera were prepared by the inoculation of 0.5 ml of a 10 ml/litre dilution of infected allantoic fluid intranasally into a lightly anaesthetized ferret. Animals were bled 14 days later and the sera treated with receptor-destroying enzyme to remove non-specific inhibitors.

Monoclonal antibodies

Two series of monoclonal antibodies were prepared, one with A/USSR/90/77(H1N1), and the second with A/Bangkok/1/79(H3N2). The method used for the production of these monoclones was based on that described by Koprowski et al. (*5*). A similar method was followed for the preparation of monoclones to A/England/333/80 (H1N1) with some modifications, i.e., the use of fresh mouse thymocytes as feeder layers for building up early clones, and limit dilution rather than separation in agar gel to ensure the monoclonal origin of the hybridomas. Antibody-producing clones were inoculated into pristane-treated mice to induce ascites and the resulting ascitic fluids stored frozen at −20 °C. These were treated with receptor-destroying enzyme before use in HI tests.

Haemagglutination-inhibition tests

HI tests were done using standard procedures, as described elsewhere (*6*).

RESULTS

Influenza A H1N1 subtype

The H1N1 subtype of influenza A virus reappeared as an epidemic strain in 1977 in China. Within a year, viruses like the prototype A/USSR/90/77 had been isolated in countries all over the world.

In 1978, A/Brazil/11/78, the first variant of the prototype, was detected in South America; this variant was encountered increasingly in the northern hemisphere in the winter of 1978–79. It could be identified by its lower reactivity with ferret antisera to the prototype and by its failure to react with certain monoclones prepared with A/USSR/90/77 (Table 1). Of the various monoclones derived from A/USSR/90/77, two (264/2 and 110/1) were selected for regular use to differentiate the variant A/Brazil/11/78 from A/USSR/90/77. Two further monoclones, 18/1 and 22/1, were later included when variants began to appear that failed to react with the monoclones 264/2 and 110/1. Table 1 also shows the numbers of each variant, as differentiated by the monoclones, found in the first four years of prevalence of the H1N1 virus.

In the winter of 1977–78, most isolates in the United Kingdom were like A/USSR/90/77, but a significant proportion were seen retrospectively to be like the variant A/Brazil/11/78. In the second year, 1978–79, isolates began to appear that did not behave with the monoclones like either the prototype or the first variant. In the third year, 1979–80, there was little H1N1 influenza in the United Kingdom, but of the 12 viruses isolated, 8 were like this last variant, which was designated A/England/333/80. In Bulgaria and Madagascar, A/USSR/90/77 was again isolated that year, whereas in Austria, China, France, and Norway, A/Brazil/11/78 viruses predominated.

In the following year, 1980–81, the variant A/England/333/80 predominated in the United Kingdom and was found in the Federal Republic of Germany, Italy, Netherlands and Switzerland, as well as in Australia, India, Japan, South Africa, and Thailand. In 1981–82, few isolates were examined, but as well as viruses like A/England/333/80, a further variant was detected by its failure to react with the monoclone, 18/3, which had previously reacted with all isolates. This variant was later designated

Table 2. Cross-reactivity of H1N1 viruses with ferret antisera in haemagglutination-inhibition tests

Virus	Ferret antiserum											
	1	2	3	4	5	6	7	8	9	10	11	12
1. A/USSR/90/77	1280	160	640	80	40	80	80	< 40	160	40	160	< 40
2. A/Brazil/11/78	160	160	80	40	< 40	40	< 40	< 40	40	40	40	< 40
3. A/England/333/80	320	160	640	320	160	160	80	80	160	160	320	< 40
4. A/Hong Kong/2/82	80	80	160	640	80	320	80	80	160	160	320	40
5. A/India/6263/80	< 40	80	320	320	640	320	160	80	80	80	320	40
6. A/Chile/1/83	< 40	< 40	80	160	40	160	40	80	40	80	160	< 40
7. A/England/530/83	< 40	40	80	160	40	80	1280	320	640	640	640	640
8. A/Texas/29/82	< 40	40	80	160	80	160	640	640	80	640	320	1280
9. A/Victoria/7/83	320	160	640	1280	320	640	1280	320	5120	640	2560	320
10. A/Bordeaux/3240/83	< 40	< 40	80	160	< 40	80	80	< 40	80	1280	160	40
11. A/England/414/83	< 40	< 40	< 40	40	< 40	40	320	160	40	160	160	320
12. A/Dunedin/27/83	< 40	< 40	< 40	40	< 40	< 40	640	320	40	640	160	1280

A/Texas/29/82. By this time, monoclones prepared with A/England/333/80 were available and in the next year this series replaced those prepared with A/USSR/90/77.

In the two seasons 1982–83 and 1983–84, influenza A(H1N1) viruses were frequently isolated in the United Kingdom and many other countries. Their cross-reactivities, as determined with ferret antisera, are shown in Table 2. The reduced reactivity of these isolates with antisera to the earlier strains of the H1N1 subtype is clear, but the precise identification of isolates from these results is difficult. When isolates were tested with monoclones prepared with A/England/333/80, it was found that isolates like A/England/333/80 still formed over one-third of the total, while the remainder reacted with several different patterns. One-quarter were like the variant detected in the previous winter of 1981–82, A/Texas/29/82. One-quarter were completely non-reactive with all the monoclones and were designated A/England/414/83; a similar variant, A/Dunedin/27/83, was isolated in New Zealand later the same year.

Between the fully reactive and the fully non-reactive, several groupings could be defined where collections of isolates failed to react with one or other of the monoclones. These groupings are shown in Table 3 with the monoclones arranged in order of decreasing reactivity. The older prototypes all fall into group 1.

The heterogeneity of viruses isolated in 1982–83 is apparent, although many of them are close to older variants like A/England/333/80. In the next year, 1983–84, strains like A/England/333/80 were already becoming rare and a drift towards non-reactivity with the monoclones could be observed. However, the anticipated predominance of strains like A/Dunedin/27/83 (Group 6) has not occurred as yet.

Influenza A H3N2 subtype

Antigenic drift in the H3N2 subtype of influenza A virus has proceeded steadily but undramatically for several years, since the appearance of A/Texas/1/77. The H3N2 viruses isolated in the years immediately after 1977 were either like that virus, or like the variant A/Bangkok/1/79, or intermediate between the two.

Monoclones prepared with A/Bangkok/1/79 did not contribute significantly to the antigenic analysis of these viruses, although the identity of an infrequent but regular variant, A/Bangkok/2/79 could be confirmed by the low titres obtained with certain of the monoclones.

In the winter of 1982–83, although most (85%) of the H3N2 viruses were of the sort described above, a small proportion were clearly different in their reactivities both with ferret antisera and with monoclones. Table 4 shows that isolates could be placed in one of four groups according to how they reacted with the monoclones. Those in group 1 were close to the fully reactive isolates, failing to be

Table 3. Groupings of H1N1 strains according to HI pattern with monoclones to A/England/333/80 (H1N1)

Group	Marker strains	Monoclone						
		B4	B6	B16	B3	B1	B2	B7
1	A/USSR/90/77 A/Brazil/11/78 A/England/333/80 A/Hong/Kong/2/82	++[a]	++	++	++	++	++	++
2	A/India/6263/80 A/England/530/83 A/Chile/1/83	++	++	+	+	+	++	+
3	A/Texas/29/83	++	+	−	+	+	++	+/−
4	A/Victoria/7/83	++	++	++	+	+	−	−
5	A/Bordeaux/3246/83	++	++	++	+	−	−	−
6	A/Dunedin/27/83 A/England/414/83	−	−	−	−	−	−	−

[a] − = < 100; + = 100–800; + + = ⩾ 1600.

Table 4. Groupings of H3N2 strains according to HI pattern with monoclones to A/Bangkok/1/79

Group	Strain	Monoclone													
		105	4/1	7/1	23/1	49/1	85/1	88/1	50/1	54/6	78/2	46/2	67/1	31/5	38/6
	A/Bangkok/1/79	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	A/Eng/7/83	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1	A/Eng/38/82	+	+	+	+	+	+	+	+	+	+	+	+	−	+
2	A/Eng/945/82	+	+	+	+	+	+	+	+	+	+	+	+	−	−
	A/Eng/943/82	+	+	+	+	+	+	+	+	+	+	+	−	−	−
	A/Eng/950/82	+	+	+	+	+	+	+	+	+	−	−	−	−	−
	A/Eng/32/83	+	+	+	+	+	+	+	+	+	−	−	−	−	−
	A/Philippines/82	+	+	+	+	+	+	+	+	−	−	−	−	−	−
	A/Eng/951/82	+	+	+	+	+	+	+	+	−	−	−	−	−	−
	A/Eng/5/83	+	+	+	+	+	+	+	+	−	−	−	−	−	−
3	A/Vic/205/82	−	−	−	−	−	−	−	−	+	+	+	+	+	+
	A/Eng/34/83	−	−	−	−	−	−	−	+	+	+	+	+	+	−
	A/Eng/64/83	−	−	−	−	−	−	−	+	+	+	+	+	+	−
	A/Eng/80/83	−	−	−	−	−	−	−	+	+	+	+	+	+	−
4	A/Eng/35/83	−	−	−	−	−	−	−	−	+	−	−	−	−	−
	A/Eng/54/83	+	−	−	−	+	−	−	+	+	+	−	−	−	−
	A/Eng/947/82	−	−	−	−	−	−	−	−	−	−	−	−	−	+

[a] − = < 100; + = 100–51 200

inhibited by only one monoclone. A larger group 2 contained A/Philippines/2/82, a variant that had been responsible for outbreaks of influenza the previous year in east Asia. A completely different pattern was produced by A/Victoria/205/82, isolated in Australia around the same time and forming the prototype of group 3; group 4 contained viruses that reacted with only a few monoclones. Viruses like A/Philippines/2/82 were the most common of the low reactors with ferret antisera but still formed only a small proportion of the H3N2 viruses examined.

In the winter of 1983–84, the H3N2 subtype was only occasionally detected worldwide, but of the viruses isolated, 70% were like A/Philippines/2/82 (group 2) and the remainder, much less reactive with monoclones, fell into group 4.

DISCUSSION

Monoclonal antibodies have already proved of great value in the study of influenza, providing information on antigenic sites on the haemagglutinin through the production of mutants growing in the presence of sharply specific antibodies. As regards the antigenic analysis of field strains, they have generally been useful only to those workers with access to sufficient numbers of isolates to enable a significant pattern of reactivity to be defined. The availability of monoclonal antibodies by no means obviates the use of more broadly reactive, polyclonal antisera, in particular ferret antisera. This animal, which is susceptible to influenza, can be infected intranasally and within two weeks will provide a convalescent antibody specific for the infecting strain. The speed with which such sera are produced cannot be matched by the system for producing hybridomas, and as the polyclonal antibody cross-reacts with other variants of the same subtype, a preliminary and sometimes definitive identification can be achieved rapidly. However, in interpandemic periods with slow and irregular antigenic changes occurring in the viruses, ferret sera often do not differentiate the variants that appear and an improved means of defining their identity has been needed for some time.

The application of monoclonal antibodies to the antigenic analysis of circulating influenza viruses was not initially found to contribute greatly to the resolution of the problem. This may have been

because in the selection of clones during the preparation of influenza hybridomas, the viruses used to test the clones were those against which the hybridomas were prepared or to earlier variants. Experience with anticipating antigenic change is still limited and, in our studies, the monoclones have become of most use prospectively. As new variants appear in successive seasons, they show increasing non-reactivity with the monoclones. Whether this non-reactivity necessarily indicates antigenic changes in the virus is not confirmed, but evidence suggesting that this is the case has been presented by Daniels et al. (*7*), who have demonstrated changes in the base sequences of the haemagglutinin that could correspond to the changes in reactivity with monoclonal antibodies described here.

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RÉSUMÉ

UTILISATION DES ANTICORPS MONOCLONAUX POUR L'ANALYSE ANTIGÉNIQUE DES VIRUS GRIPPAUX A

L'analyse antigénique des virus grippaux A dans l'intervalle des pandémies est compliquée par les modifications lentes et irrégulières subies par les virus. Pour vérifier s'il existait chez ces variants des caractéristiques régulières que les anticorps polyclonaux n'étaient pas capables de révéler, on a inclus des préparations d'anticorps monoclonaux dans les épreuves courantes d'inhibition de l'hémagglutination utilisées pour caractériser les virus isolés.

Des anticorps monoclonaux dirigés contre le virus A/USSR/90/77 prototype de H1N1 se sont montrés capables de différencier ce virus du variant A/Brazil/11/78. Au cours des années ultérieures, des anticorps monoclonaux dirigés contre A/England/333/80 ont été utilisés pour identifier une série de variants qui ont pu être groupés selon leur mode de réactivité à l'égard de ces anticorps monoclonaux.

Une méthode similaire, appliqué aux virus H3N2, a montré que les variants de ce sous-type pouvaient également être regroupés en fonction de leur réactivité en présence d'anticorps monoclonaux préparés avec A/Bangkok/1/79. Des variants tels que A/Philippines/2/82 pouvaient être nettement différenciés par ces anticorps monoclonaux.

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