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Monoclonal Antibodies: Implications for Virology

Brief Review

By

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With 9 Figures

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Introduction

It is now nine years since KOHLER and MILSTEIN (65) first produced hybridoma cell lines secreting monoclonal antibodies (MAb) of a predefined specificity. In 1977/78, KOPROWSKI and co-workers (36, 67, 125) pioneered the production of MAb against viruses and viral antigens. MAb have now been produced against representative members of most taxonomic virus groups. This subject has been adequately reviewed elsewhere (18, 98, 126, 127, 130) and it is not the intention of this article to elaborate along these lines. Instead, certain implications of the use of MAb in virology which are directly related to the interaction of antibody with virus and the result of that reaction will be introduced and discussed. The major areas for discussion will be 1. valency of the MAb; 2. virus neutralisation by the MAb; 3. *in vivo* functions of MAb; 4. affinity, specificity and paratope/epitope interactions with MAb; and 5. idiotopes (Id) and Id-anti Id interactions. These are the fields of study which are of relevance to the immunological defence mechanisms *in vivo*, and as such can provide information which may be of use in the control of virus infection. Other areas, such as epitope identification and topographical mapping, and antigenic modulation can, in turn, be related to that virus-antibody reaction and the immune response against the virus.

MAb have also been used preparatively, for instance in affinity chromatography. It is not intended to review this topic here since generalisations of the procedure are impractical, and it should be left to the individual to determine whether or not a particular MAb can be so used. One other large area of application of MAb in virology has been in diagnosis/epidemiology/

strain differentiation. These topics require a separate review, which has been suitably presented by other authors, such as OXFORD (98), YOLKEN (128) and CARTER and TER MEULEN (18).

The objective of this article is to present data obtained using MAb which can be of use in the study of immunological protection against virus infection. Selected articles will be discussed in detail where they have provided substantial new information in this area, and possible implications of this work for virological studies will be proposed.

Valency of MAb

Myeloma cells (MC) fuse preferentially with B lymphocytes and lymphoblasts (66) and can enhance the capacity of these leucocytes to secrete antibody (89). The MC can also influence the B cell genes at the phenotypic level, as a consequence of the secretory characteristics of the MC. This will determine the valency and hence affinity of the subsequently produced antibody for a particular epitope. The valency of an antibody refers to the number of sites on the molecule which can bind to antigen. An antibody molecule consists of two heavy and two light chains which combine through disulphide bridges to form a single F_c region and two Fab regions (see Figs. 1, 3 and 9). At the end of the Fab region furthest from the F_c is the paratope (Fig. 1) or antigen-binding site. If each Fab has an identical paratope, the antibody is bivalent in reactivity for the homologous antigen; if the Fab have different paratopes, the antibody is monovalent (Fig. 1).

Table 1 shows the MC lines in most common use today. (The human MC shown are an example of the apparently stable lines reported. The influence they have over the antibody production of a hybridoma is similar to that of mouse MC lines.) With hybridomas between an antigen-specific splenocyte secreting a heavy (H) and light (L) chain, and the X63 MC which can secrete the MOPC21 heavy (γ) and light (κ) chains it is theoretically possible to have secretion of both splenocyte and MC Ig chains, either as separate

Table 1. *Myeloma cell lines currently in use with hybridoma technology, and their characteristics of antibody heavy chain (γ) and light chain (k) synthesis*

Cell line	Animal source	Synthesis	Secretion
P3-X63Ag8 (X63)	Balb/c mouse	γ_1k	γ_1k
P3-NSI/1-Ag4.1 (NS1)	Balb/c mouse	k	—
P3-X63-Ag8.653 (NS653)	Balb/c mouse	—	—
SP2/0-Ag14 (SP2/0)	Balb/c mouse	—	—
NSO/u	Balb/c mouse	—	—
Y3-Ag1.2.3 (Y3)	Rat	k	k
YB2/3.0Ag0 (YB2)	Rat	—	—
GM1500 6TG-A11	Human	γ_2k	γ_2k
GM1500 6TG-A12	Human	γ_2k	γ_2k

entities or as recombinant molecules. Assuming random distribution laws apply, the various recombinant molecules which could arise and their relative frequencies are shown in Tables 2 and 3. As can be seen, the hybridoma cells have a higher probability of producing monovalent than bivalent antigen-specific antibody molecules.

Table 2. *Possible recombinant Ab molecules in hybridomas of splenocytes and the P3-X63-Ag8 myeloma cell line, assuming random distribution, and equivalent likelihood of both homologous and heterologous heavy chain and light chain re-assortment*

Frequency	Ab chains	Valency	Ag-Specific
1/36	H_2L_2	2	+
	H_2Lk		
16/36	H_2Lk	1	+
	? $H\gamma L_2$		
	? $H\gamma Lk$ (HLγk)		
19/36	γ_2k_2	2	—
	γ_2Lk	1	
	γ_2L_2	2	
	? $H\gamma k_2$	1	
	? $H\gamma Lk$ (HkγL)	1	

H, L represent splenocyte heavy and light chains respectively
γ, k represent X63 heavy and light chains respectively

Table 3. *Comparative secretory potential of hybridoma cultures and autologous splenocytes in Jerne haemolytic plaque-forming cell assays for both antigen-specific antibody and total immunoglobulin*

Source of Ig	Ig	% Cells secreting that Ig
Hyperimmune spleen	Ag-Specific	1
Hybridomas	Ag-Specific	10
Hyperimmune spleen	All specificities	5
Hybridomas	All specificities	25—90[a]

[a] Depending on the source of spleen cells and line of myeloma cells used

This can be seen more clearly with the recombinant molecules from hybridomas produced using antigen-specific splenocytes and the NS1 MC line (Fig. 1). Since NS1 cells synthesise but do not secrete a MOPC21 $\varkappa$ (light) chain, this $\varkappa$ chain will only be found in culture supernatants as part of a hybrid Ig molecule. Assuming random distribution, 25 per cent of the antigen-specific MAb produced from such a fusion will be bivalent molecules (i.e. they will have the "splenocyte phenotype" H_2L_2). In contrast 50 per cent of the antigen-specific MAb will be monovalent ($H_2\varkappa L$

phenotype). The remaining 25 per cent of such MAb will be non-reactive against the relevant antigen since both heavy chains will be in association with MC-specified $\varkappa$ chains ($H_2\varkappa_2$ phenotype).

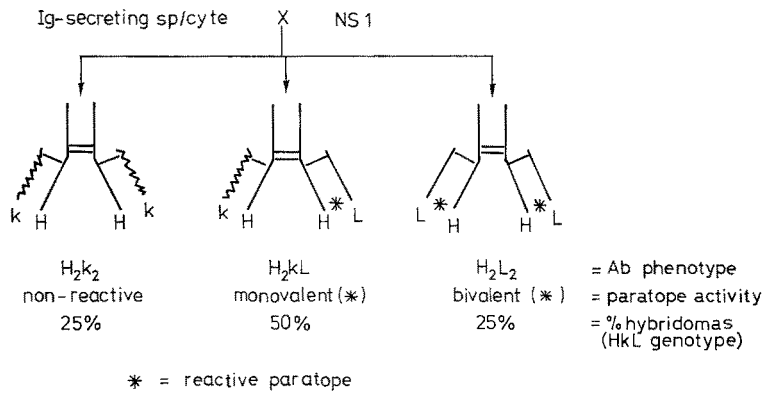

Fig. 1. Antibody synthesis by hybridomas utilising NS 1 myeloma cells as a parent. The theoretical distribution of antibody phenotypes from hybridomas formed between splenocytes (Sp/cytes) synthesising a heavy "H" chain and a light "L" chain, and NS 1 cells synthesising a light "k" chain. * the paratope formed between the H and L chains which is reactive against the immunising antigen

Both bivalent and monovalent MAb can therefore be used to determine the characteristics of antibody reaction with particular epitopes. That is, they can be used to study the mechanisms of virus neutralisation (in the presence or absence of complement), *in vivo* passive protection against virus challenge, and the relative affinity of the MAb-virus interaction. Conclusions drawn from work with MAb must therefore take into consideration the different immunochemical properties of monovalent and bivalent antibodies (reviewed by MANDEL, 76).

Virus Neutralisation by MAb

The various mechanisms by which antibody could directly or indirectly effect *in vitro* neutralisation of virus infectivity have been extensively reviewed by MANDEL (76, 77), COOPER (21), OLDSTONE (93, 94), SISSONS and OLDSTONE (112) and DIMMOCK (27). In his review, DIMMOCK also discusses the data reported on virus neutralisation using MAb. MANDEL proposed several mechanisms of neutralisation (intrinsic, extrinsic and pseudoneutralisation), but DIMMOCK simplified the picture by considering virus neutralisation as an inhibition of either adsorption, penetration, or a post-penetration step. Depending on the antibody, virus or cell system used, different authors have reported neutralisation through the inhibition of one of these processes. Inhibition of adsorption may be through the binding

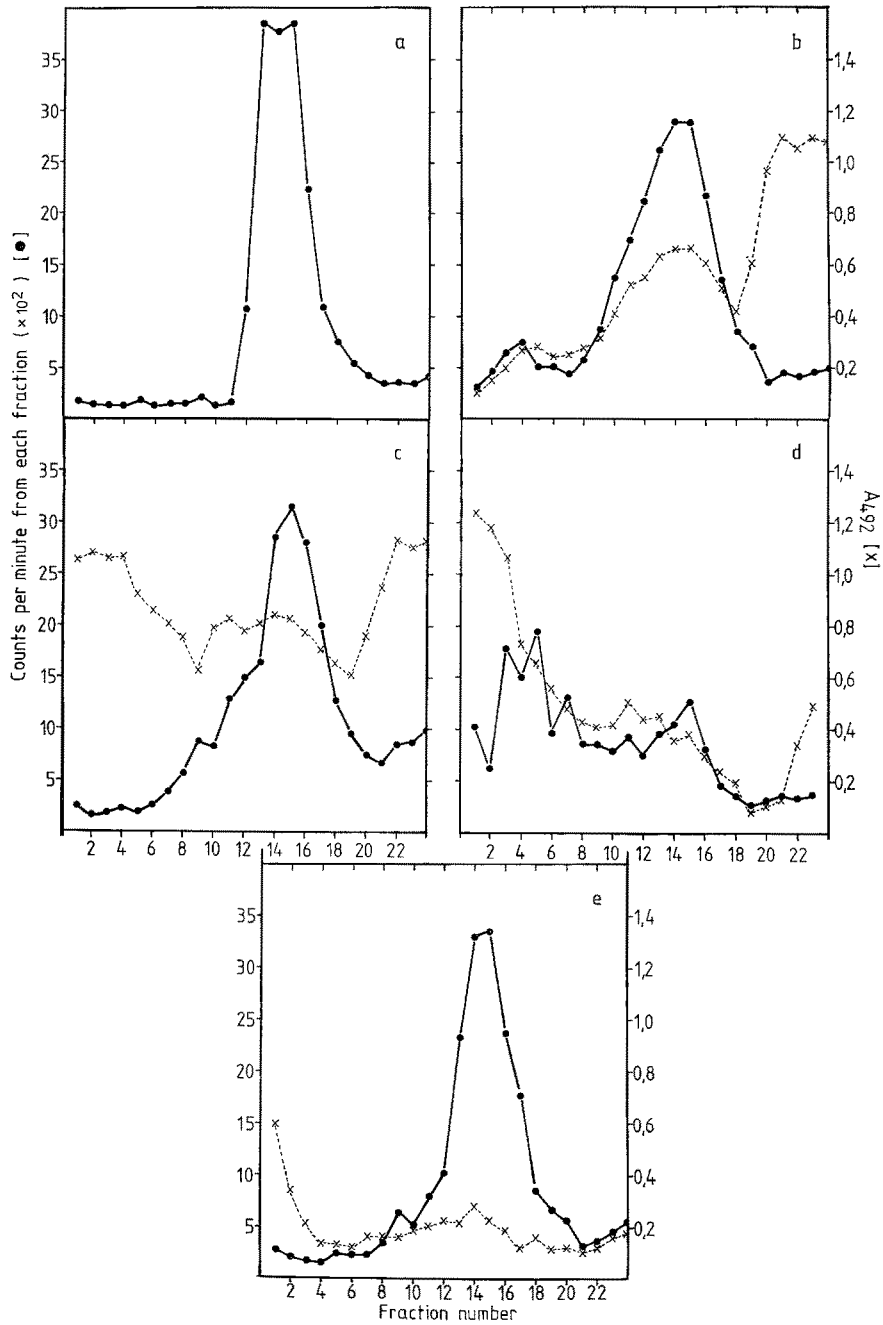

Fig. 2. Analysis of radioactive counts associated with the fractions from sucrose density gradients (85) of ^{35}S-methionine labelled foot-and-mouth disease virus (whole virions — 146S antigen) before (a) and after (b—e) reaction with neutralising concentrations (b—d) and non-neutralising concentrations (e) of MAb. *a* Virus alone; *b* virus + 10^{-1} dilution of MAb; *c* virus + 10^{-2} dilution of MAb; *d* virus + 10^{-3} dilution of MAb; *e* virus + 10^{-4} dilution of MAb. • cpm; × antibody concentration (A_{492} from sandwich ELISA for determining antibody concentration)

of antibody at or near the cell attachment site on the virus; "bridging" of this cell binding site through the MAb reacting with epitopes on either side of it; saturation (envelopment) of the virion surface by antibody; aggregation of virus particles to form virus/antibody complexes. Monovalent and bivalent MAb should help to discriminate these mechanisms since only the bivalent molecules can neutralise by "bridging" or aggregation of virus particles. DIMMOCK (27) has reviewed the evidence concerning the relative efficacy of complete antibody molecules and their Fab fragments in the neutralisation of influenza virus and poliovirus infectivity.

Both polyclonal antisera (reviewed by MANDEL, 76, 77) and MAb (5, 30, 42, 62, 85) can neutralise virus through an inhibition of adsorption to susceptible cells. Whether or not this neutralisation was due to direct or indirect blocking of the cell binding site on the virus is unclear. Only the relationship of aggregation of virus particles to neutralisation has been studied in any detail (1, 2, 7, 46, 69, 95, 124). BRIOEN *et al.* (12) related the size of poliovirus/MAb complexes to the efficiency of neutralisation, and BAXT *et al.* (5) showed that MAb which neutralised foot-and-mouth disease virus (FMDV) also aggregated virions. However, aggregation of virus by MAb will not always prevent infection of the cell (see DIMMOCK, 27). For example aggregation of FMDV by MAb could not always be related to neutralisation (5). In fact, work by McCULLOUGH and co-workers (85) has shown that MAb can neutralise FMDV without a requirement for aggregation (Fig. 2). The figure shows sucrose density gradient profiles of ^{35}S-methionine labelled FMDV before and after reaction with a MAb — 4C9 — at neutralising (b—d) and non-neutralising (e) concentrations. Substantial aggregation of virions was found at the 10^{-3} dilution of antibody, but at the 10^{-1} dilution the majority of neutralised virus antigen and MAb was in small complexes which sedimented in a similar position to unreacted virus.

MAb can also inhibit virus replication at a post-penetration stage (27). This may be reflecting antibody-induced conformational changes in the virus. With influenza viruses, such changes are induced; the neutralisation being related to an impairment of transcription. But the work with poliovirus suggests that different MAb can neutralise virus by different mechanisms, or a single MAb can neutralise by a number of alternative procedures.

ICENOGLE *et al.* (48), using the experimental design shown in Fig. 3 demonstrated that five-times more Fab fragments than complete 7S IgG molecules were required to neutralise poliovirus. The authors related cross-linking of the virus by antibody to neutralisation. However, in a MAb/neutralised virus mixture, only a portion of the virus was aggregated. Furthermore, the 3.5S Fab fragments (which cannot cross-link virions) neutralised virus, albeit less efficiently. From this, it is possible that the neutralisation was also effected by antibody-induced conformational changes

in the virus and the reduced efficacy of Fab fragments compared with 7S antibody was due to the lower affinity of the former. [PHILIPSON and BENNICH (100) showed that the induction of conformational changes in viruses was more efficient with bivalent antibody than with the monovalent 3.5S fragment.]

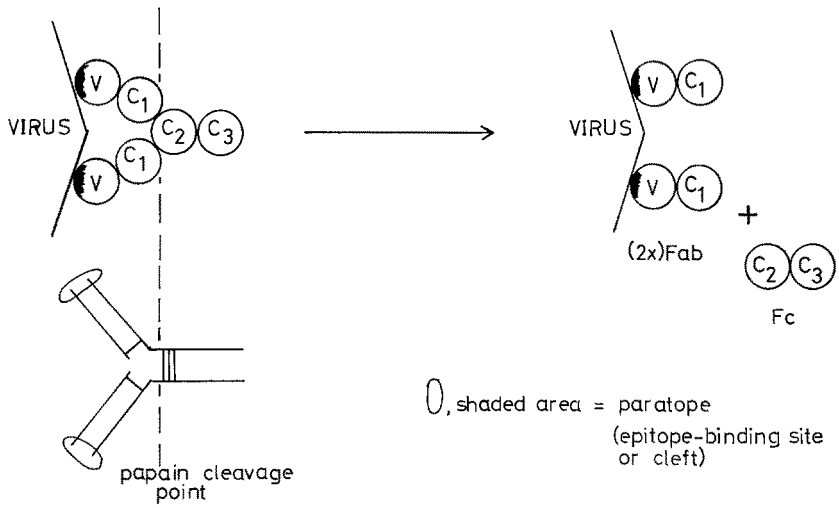

Fig. 3. Effect of papain treatment on a MAb after reaction with virus. *O* paratope on the line drawing of the MAb. Shaded area, paratope on the domain drawing of the MAb. *V, C* represent the variable and constant domains, respectively, of the MAb

EMINI *et al.* (30) demonstrated that some of their MAb neutralised poliovirus by inhibiting adsorption, whereas others inhibited virus RNA synthesis with little effect on adsorption. A number of their MAb neutralised some of the particles in a virus preparation by inhibiting adsorption, and neutralised other particles by inhibiting a post-penetration stage. In all cases, antibody-induced conformational changes in the virus were detected. Thus, MAb can neutralise poliovirus by aggregating virus particles, bridging or destroying the conformation of epitopes essential for adsorption of virus to host cells, and inhibiting a post-penetration stage of virus replication through the induction of conformational changes in the virion. Different MAb do not always neutralise by the same mechanism, but at least some of the MAb can use more than one procedure.

We have also shown that MAb against FMDV can inhibit either adsorption or a post-penetration event, but perhaps the most significant observation was the mechanism of neutralisation by a MAb which reacted in the centre of each virion face (85). The antibody induced conformational alterations in the virus such that they lost their RNA genome and appeared as "empty particles" under the electron microscope (Fig. 4). This neutrali-

sation was irreversible, which is a contrast to some of the work cited by COOPER (21) on virus neutralisation by polyclonal antisera. It is possible, therefore, that irreversible virus neutralisation by antibody requires the induction of conformational changes such as those in Fig. 4.

Synergistic reactions between MAb can also result in the neutralisation of virus infectivity. This has been reported using bovine leukemia virus (14), influenza A virus (75), hepatitis B_e antigen (49), VSV (122), Sindbis virus (20), and La Crosse virus (62). KINGSFORD *et al.* (63) also reported that non-neutralising MAb against La Crosse virus enhanced the binding of neutralising antibody. These examples may represent conformational changes induced by one MAb altering epitope expression by the virus so that the second MAb binds or neutralises more efficiently (reviewed by DIMMOCK, 27). A relationship between the reactivity of MAb and induced conformational alterations in the virus has been shown by AL MOUDALLAL *et al.* (3) with tobacco mosaic virus and MCCULLOUGH *et al.* (82, 83) with foot-and-mouth disease virus. Since synergistic reactions between antibodies will probably occur in an antiserum, MAb may permit closer scrutiny of these reactions, and their influence on both *in vitro* and *in vivo* effects of antibody on virus .

In addition to demonstrating different mechanisms of neutralisation, MAb have also identified distinct epitopes on several viruses. Using competition studies between MAb to determine the relatedness of the epitopes with which they react, distinct neutralisable epitopes have been identified on mouse mammary tumor virus (78), bovine leukemia virus (15), herpes simplex virus (103), VSV (122), feline leukemia virus (40), La Crosse virus (62, 63), rabies virus (70), Sindbis virus (109), murine hepatitis virus (16), Coxsackie virus (17), Newcastle disease virus (50) and FMDV (83). Differentiation of neutralisable epitopes has also been attempted by comparing the reactivity of MAb against different strains of the appropriate virus (30, 51). Some viruses have been found to carry only a single neutralisable epitope on their virions (104). The identification and topology of neutralisable and non-neutralisable epitopes on viruses using MAb has been reviewed in detail by CARTER and TER MEULEN (18) and POLLOCK *et al.* (101). Some of these epitopes have been related to maturation events in virus assembly (for example, 29, 80).

In summary, MAb (both monovalent and bivalent) have revealed considerable information about virus neutralisation at the single molecule

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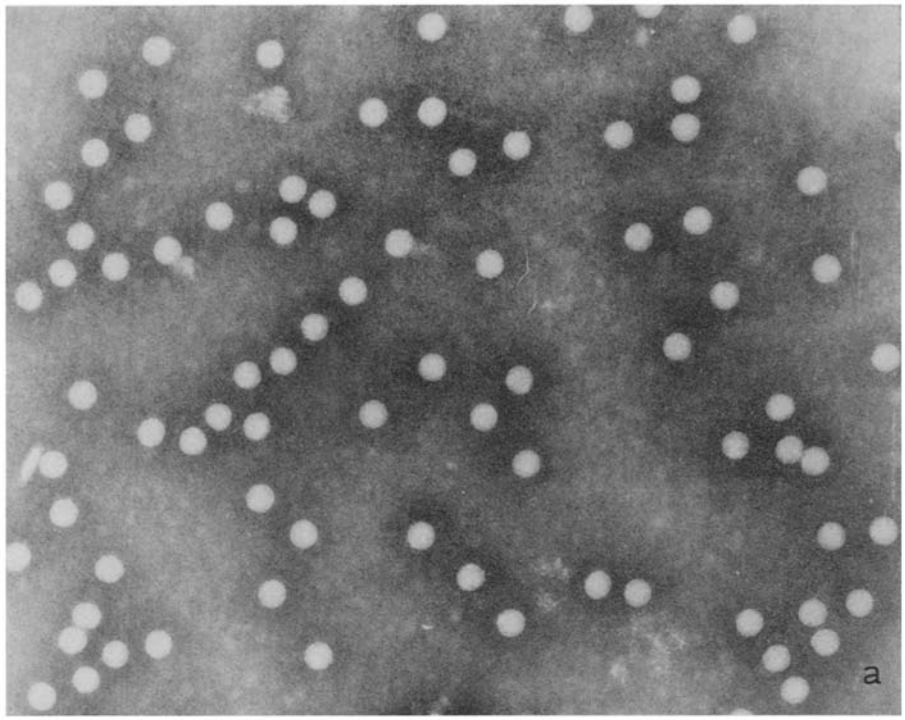
Fig. 4. Electron micrographs of foot-and-mouth disease virus before (*a*) and after (*b*) neutralisation of infectivity by MAb 4C9. Thirty μg purified infectious virus (146S) were incubated with a 10^{-2} dilution of MAb, which inhibited >99 per cent plaque formation, for 1 hour at 37° C. The mixture was then applied to thin film grids, stained with phospho-tungstic acid, and viewed under an electron microscope as described elsewhere (85)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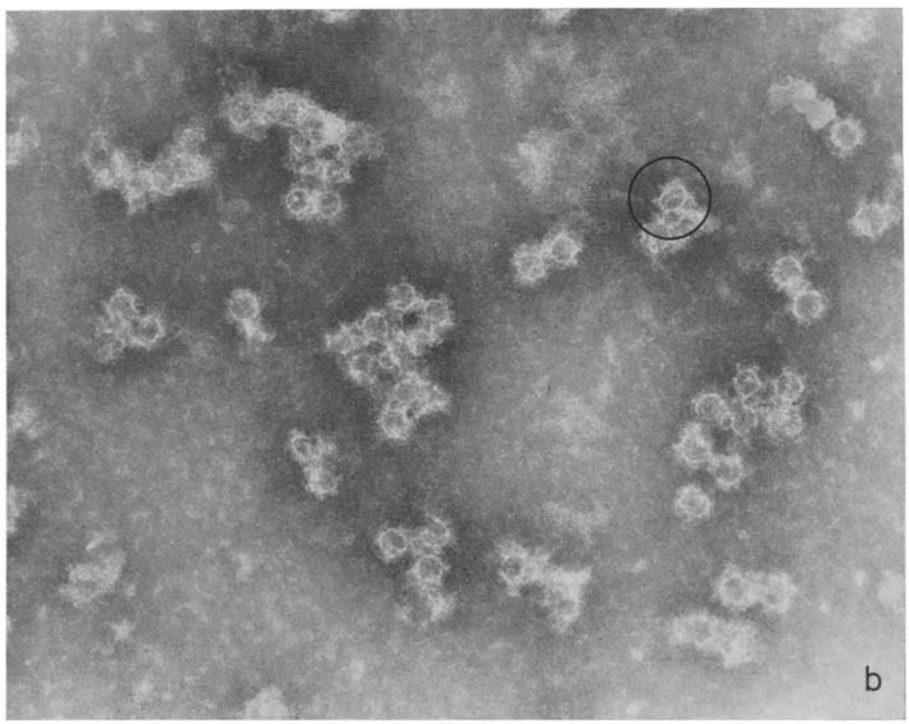
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level. It is apparent that neutralisation can be effected through the inhibition of either virus adsorption or a post-penetration event. Also, antibodies derived from different fusions will not neccesarily produce similar results to one another since the ability of 7S IgG, $F(ab')_2$ and Fab fragments to neutralise herpesvirus varied with the time after immunisation at which the IgG was obtained (111). It remains to be determined if each mechanism of virus neutralisation has equivalent relevance *in vivo*. In fact, antibody-mediated inhibition of virus replication may be of secondary importance to the immunological defence mechanisms which attack antibody/virus complexes — complement-mediated functions, opsonisation and antibody-dependent cellular cytoxicity (ADCC) reactions. To study these immune processes, the MAb must have an F_c moiety. Within this region of the antibody molecule lie the sites for complement (C′) activation and F_c receptor-bearing (F_cR^+) cell binding (required for opsonisation and ADCC). Both monovalent and bivalent reactions could again be compared, but for the former, MAb derived from the appropriate hybridomas of NS1 myeloma cell parentage would be required. These would retain the F_c moiety (unlike the Fab fragments generated by, for example, ICENOGLE *et al.* 48), and would not have been subject to enzyme digestion.

The Influence of Complement on Neutralisation of Viruses by MAb

There are two pathways for the activation of complement — the classical pathway and the alternative pathway. The function of these in humoral immunity against viruses has been reviewed by COOPER (21), OLDSTONE and LAMPERT (97), SISSONS and OLDSTONE (112). The activation of the full complement cascade results in membrane attack (a typical example of this is shown by HUMPHREY and WHITE, 47). This can effect irreversible damage to either the envelope of enveloped viruses (93, 97, 112) or the plasma membrane of virus infected cells in which viral proteins or infection-associated proteins have been inserted. However, complement-effected lysis will not necessarily follow from complement activation. Firstly, if antibody reacts with an epitope near the extremity of prominent surface projections on the virus the activated $C4_b$, $C3_b$ or $C5_b$ component of the complement cascade may lose its ability to bind to the membrane before it can diffuse from the site of activation (MAb-epitope interaction) to the membrane (21). Secondly, complement-mediated membrane damage may occur but fail to produce lysis if the antibody binds to low density epitopes on the virus or infected cell. If this density is too low, the cell can recover and repair the damage. Finally, antibody may fail to activate complement if the ratio of antibody: antigen is too low to overcome the natural inhibitory factors present in serum (21, 45). The failure of antibody to activate complement mediated lysis may be masked in polyclonal antisera by the effective

antibody populations present. This would not be the case when using the appropriate MAb.

Complement can also neutralise infectious complexes of virus and antibody, or enhance neutralisation by the antibody, without activation of the full cascade (Fig. 5). With the majority of viruses studied, complement-enhanced virus neutralisation (first demonstrated with vaccinia virus by GORDON, 39) and complement-dependent immune defence *in vitro* and *in vivo* is effected mainly by the alternative pathway (21, 76, 77, 112) although there are exceptions in which the classical pathway is dominant, e.g. African swine fever (92).

Complement-enhanced virus neutralisation by MAb has been reported by FORGHANI *et al.* (32), RECTOR *et al.* (103), HOLLAND *et al.* (43), KIMURA-KURODA and YASUI (61) and RUSSELL *et al.* (106). Since certain MAb will only neutralise virus in the presence of complement (32, 43, 61) it is possible that such MAb alone cannot induce sufficient conformational changes in the virus to effect neutralisation.

Complement can also also enhance or effect virus neutralisation by envelopment of the virus or aggregation of virus-antibody complexes. It probably inhibits virus adsorption/penetration by steric interference (6, 25, 95, 123, 124). Conversely, complement can *solubilise* virus/antibody complexes (88). BRIOEN *et al.* (12) have reported that the infectiousness of their MAb/poliovirus complexes decreased with size of the complex. However, WALLIS and MELNICK (124) noted that the virus in their HSV-antibody complexes was not neutralised but was behaving as a single infectious unit due to the aggregation (infectious complex). If a MAb aggregated virus to produce infectious immune complexes (decreased the virus infectivity titre as measured *in vitro*) such complexes could be reduced in size by complement solubilisation *in vivo*, and the virus infectivity titre increased. Since the MAb/virus complex had remained infectious, the MAb alone cannot be

Table 4. *Comparison of the capacity of anti-O_1 Switzerland 1965 FMDV monoclonal antibodies to neutralise virus infectivity* in vitro *and passively protect 5—6 day old Balb/c mice against subsequent virus challenge, using five different FMDV type O_1 isolates from different geographical regions of Great Britain at the time of the 1967 epizootic*

	Number of mice protected/number inoculated (1_{10} virus neutralisation titre of MAb *in vitro*) using the isolates				
MAb	O_1 BFS 1781	O_1 BFS 1810	O_1 BFS 1837	O_1 BFS 1848	O_1 BFS 1860
B2	14/14 (5.5)	0/8 (0)	10/10 (5.0)	12/13 (4.8)	10/10 (5.5)
1C6	13/13 (3.5)	7/7 (3.0)	9/9 (3.5)	5/15 (2.5)	10/10 (3.8)
3C8	0/13 (1.0)	6/8 (5.5)	9/9 (5.5)	13/13 (5.0)	9/9 (6.0)
4C9	0/13 (3.5)	7/7 (5.5)	8/8 (6.2)	13/14 (6.0)	10/10 (6.0)
D9	12/14 (4.0)	1/8 (0)	7/8 (4.5)	12/13 (4.0)	9/9 (4.8)

considered an *in vivo* protective agent. BUCHMEIER *et al.* (16) with murine hepatitis virus and McCULLOUGH *et al.* (84) with FMDV identified MAb which would neutralise virus *in vitro* but not protect against virus challenge *in vivo* (Table 4). This is in contrast to antibody which can neutralise virus infectivity *per se*, and thus be effective both *in vitro* and *in vivo* (4, 71, 73, 79, 84, 103, 108, 110, 118). However, such antibodies can have a much higher neutralisation (protection) titre *in vivo* than *in vitro* which cannot be related solely to complement enhancement (84). This exemplifies the importance of antibody-dependent leukocyte-mediated neutralisation of viruses *in vivo* (opsonisation, antibody-dependent cellular cytotoxicity).

Leukocyte-Mediated Virus Neutralisation *in vivo* (Fig. 5)

When an antibody forms a complex with virus (opsonises), this can be phagocytosed by leukocytes *in vivo*. The antibody does not have to neutralise virus infectivity for opsonisation to be effective (unless the virus can replicate in the leukocytes). Similarly, the antibody does not have to activate complement to effect *in vivo* protection against a virus. In fact, not all IgG isotypes will fix (activate) complement, and there is a time dependence for the appearance after infection of complement-dependent virus-neutralising antibody. Generally speaking, the order of complement-dependence is that early IgM is the most complement-dependent, followed by late IgM, and then early IgG, while late IgG is often complement-independent (117, 119, 129, 130, 131, 132). Phagocytosis is effected through the F_c receptor (for Ag/Ab complexes) and the $C3_b$ receptor (for antigen/antibody/complement complexes) of mononuclear phagocytes and polymorphs. The complex is bound by the appropriate receptor, phagocytosed and the antigen degraded

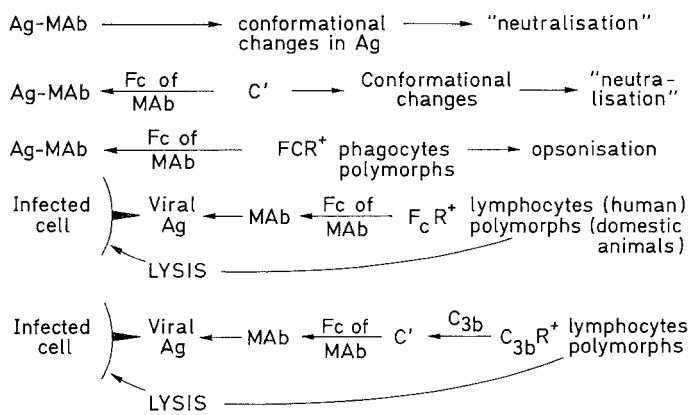

Fig. 5. Comparison of the effector pathways of virus neutralisation through MAb-induced conformational changes, C′-enhanced MAb-induced conformational changes, opsonisation (virus-MAb or virus-MAb-C′ complexes), antibody-dependent cellular cytotoxicity (ADCC), and C′-enhanced ADCC

enzymatically (Fig. 5). However, Ag/Ab complexes can effect other immunological pathways. The follicular dendritic cells (FDC) retain Ag/Ab or Ag/Ab/complement complexes within the germinal centres of secondary lymphoid organs, and present the complexes to the resident B cells and T helper cells (64), apparently resulting in memory cell development. In contrast, the F_c receptor on B lymphocytes may be involved in a negative regulatory feedback mechanism effected by Ag/Ab complexes bridging the Ig-like antigen receptor and the F_cR, thus inhibiting B lymphocyte differentiation (114, 115, 116).

Opsonisation phenomena are immune defences against cell-free virus, bacteria or other parasites complexed with antibody ($\pm$ complement). Leukocytes can also attack virus infected cells by a process equivalent to opsonisation and termed antibody-dependent cellular cytotoxicity (ADCC) and complement-enhanced ADCC (pathways 4 and 5, Fig. 5). Antibody, or antibody plus complement, may neutralise virus infectivity but be ineffective against infected cells as sources of infectious virus. Antibody alone cannot destroy virus-infected cells, while complement-mediated lysis requires a minimum density of antibody on the cell membrane. In contrast the F_cR^+ leukocytes can react with antibody or $C3_b$ bound to infected cells through their F_cR or $C3_bR$ respectively, and thus mediate lysis ("neutralisation") of the infected cells. The major effectors of ADCC have been shown to be lymphocytes (humans), monocytes (rodents) or polymorphs and macrophages (domestic animals).

Therefore, an antibody which does not neutralise virus *in vitro* may still afford protection *in vivo* (4, 9, 22, 71, 84, 103, 108). Such antibodies have provided evidence of the relative roles of the different *in vivo* immune defence processes. For example, RECTOR *et al.* (103) showed that one of four anti-HSV1 MAb passively protected mice, neutralised virus *in vitro*, both in the presence and absence of complement, and was effective in ADCC and complement-mediated lysis of infected cells. This antibody probably protects by neutralising virus infectivity, opsonisation, ADCC and complement-mediated lysis. A second and third MAb neutralised virus poorly or not at all *in vitro*, but did protect mice and had high titres in ADCC assays. The addition of complement had no effect on the *in vitro* neutralisation titre, nor could complement-mediated lysis of infected cells be induced. Thus, *in vivo* protection by these MAb was probably due to opsonisation and ADCC. The fourth MAb also passively protected mice but did not neutralise virus *in vitro* (with or without complement), and was ineffective in either ADCC or complement mediated cytolysis. Protection by this antibody may involve opsonisation, complement-enhanced ADCC, or the requirement for more than one compartment of the immune system.

Further insight into the efficiency of opsonisation of virus and subsequent enhanced phagocytosis has come from work with MAb against FMDV (84).

Table 5. *Comparison of the capacity of whole molecules and F (ab')$_2$ fragments of anti-O$_1$ Switzerland 1965 FMDV MAb to neutralise virus infectivity* in vitro *(microneutralisation test on BHK cell monolayers [23, 24]) and to neutralise virus pathogenicity* in vivo *(passive protection assay in neonatal mice [23, 81]) in preformed complexes of MAb with homologous virus*

	1_{10} *in vitro* neutralisation titre (in presence of complement)		1_{10} *in vivo* protection titre	
MAb	IgG	F (ab')$_2$	IgG	F (ab')$_2$
B2	5.5	5.0	8.0	4.5
D9	4.8	4.5	7.5	4.5
1C6	3.1	3.0	6.1	3.0
4C9	4.8	5.0	7.5	4.8
3C8	5.5	5.0	8.0	4.5

The challenge of 5 to 6 day old Balb/c mice with pre-formed complexes of MAb and homologous virus revealed a 100 to 1,000-fold increase in the capacity of the antibody to "neutralise" virus infectivity over *in vitro* assays (Table 5). When F(ab')$_2$ fragments of the antibodies were used, no *in vivo* enhancement of "neutralisation" was seen. The capacity of peritoneal exudate adherent cells to phagocytose virus (Fig. 6) was enhanced when the virus was opsonised with dilutions of MAb which could not neutralise virus infectivity by *in vitro* assays, but could prevent pathogenesis of the

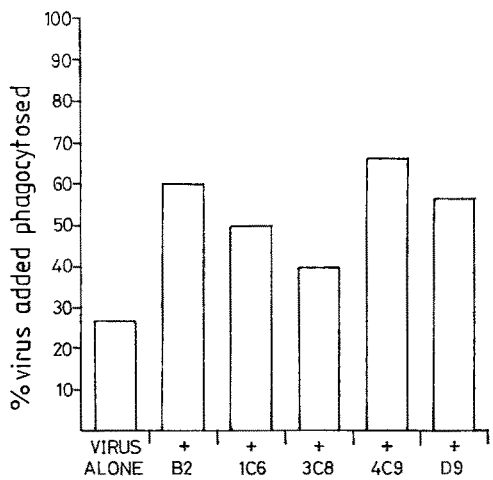

Fig. 6. Uptake by Balb/c mouse peritoneal exudate adherent cells of ^{3}H-uridine labelled FMDV 146S antigen (whole virus) either alone or after complexing with dilutions of MAb which did not neutralise virus infectivity in an *in vitro* assay, but did passively protect mice from challenge with pre-formed complexes of MAb and virus. The uptake is expressed as a percentage of the total counts added

virus in infectious immune complexes inoculated into 5 to 6 day old Balb/c mice. This effect was again abrogated by removal of the F_c moiety of the antibody. The results demonstrate that while neutralisation of virus infectivity by the MAb is an important immunological defence, opsonisation and enhanced phagocytosis in the absence of virus neutralisation is probably the major defence mechanism (ADCC, NK and CTL activities are inoperative against this virus). With viruses such as the flaviviruses and Aujeszky's disease virus which can replicate in monocytes, the opsonisation of the virus would also have to result in the neutralisation of its infectivity; phagocytosis of opsonised, non-neutralised virus would propagate the infection as opposed to destroying it.

MAb can therefore be used to determine which epitopes on the virus are related to *in vivo* protection, and what mechanisms of protection are effective. MAb can however show variation in affinity of binding with epitopes, and conclusions about the relevance of particular antibody/epitope reactions in a protective response require a knowledge of such affinities. This is also necessary for the interpretation of studies into the mechanisms of *in vitro* virus neutralisation.

Affinity and Specificity of MAb

The affinity and specificity of a MAb is dependent on the genotype and phenotype of the B lymphocyte from which the hybridoma was derived. A B cell population which has been recently stimulated by antigen will probably give rise to a MAb of lower affinity than a B lymphocyte population which has been stimulated by repeated immunizations. Furthermore, antibody-secreting hybridomas can apparently be produced from resting B lymphocytes (see the review by MILSTEIN, 89 and Table 3). However, neither the antigen-reactivity nor the affinity for a particular epitope of a series of MAb can be directly related to an autologous antiserum (the antiserum from the mice which donated their spleens for the fusion).

i) Antigen-Reactivity of MAb and Autologous Antisera

MAb derived from a particular fusion may not have the immunochemical properties of the autologous antiserum because such MAb cannot represent the whole antibody population of that autologous antiserum. Neither are the hybridomas produced representative, genotypically and phenotypically, of the spleen cell population used for the fusion (Table 3 and MILSTEIN, 89). Instead, they represent the genetic potential of both the B lymphocyte and lymphoblast complement of the spleen.

After one or two immunisations, the phenotype of the plasma cell (PC) population present in the secondary lymphoid organs will probably be reflected in the specific antibody population found in the autologous anti-

serum. In contrast with multi-boost immunisations, the relative proportions in the antiserum of antibody molecules which have resulted from previous inoculations will not necessarily be reflected in the population of B lymphoblasts in the spleen or lymph nodes. Antisera generally reflect the proportion of antibody-secreting cells, in particular the PC, in all secondary lymphoid organs which were present both at the time of bleeding and during, at least, the previous 21 days — since the half-life ($t_{1/2}$) for IgG is 21 days. That is, antisera represent the antibody production over a number of days in all lymphoid organs. MAb, on the other hand, reflect the number of *immature (excluding PC)* lymphoblastic B cells and perhaps resting B cells in the *particular* lymphoid organ used for fusion. Thus, MAb derived from single fusion are representative of an antibody population which is likely to have arisen during a short period of time from a single lymphoid organ. For these reasons the characteristics of a series of MAb will probably be unrepresentative of the antiserum of the animal which provided the spleen (or lymph nodes) for fusion. The relatedness of this series of MAb to non-autologous antisera is likely to be more distant.

ii) Affinity of MAb Compared with the Avidity of Autologous Antisera

The avidities of a group of MAb and the autologous antiserum can also be quite distinct. If that antiserum is of high avidity, this reflects the dominant reaction rate and binding constant amongst the different antibody molecules present in the antiserum. Individual antibody molecules may not exhibit such an affinity (affinity will be used to describe the binding capacity of individual and monoclonal antibodies since the reaction is between a single paratope and single epitope; avidity will be used to describe the cumulative affinities of antibody molecules as seen with antiserum).

A higher avidity may be observed with the antiserum than with the constituent antibody molecules for several reasons, not all mutually exclusive. Synergistic reactions of lower affinity molecules could enhance the binding and hence affinity of each antibody. BRUCK *et al.* (14), LUBECK and GERHARD (75), and CLEGG *et al.* (20) have demonstrated synergistic reactions between two MAb involving antibody-induced conformational changes, although affinities were not measured. Such reactions would occur in an antiserum and thus confer a higher than expected avidity on the antiserum.

However, some of the antibody molecules in an antiserum may be of similar affinity to the avidity of the serum. Such high affinity antibodies may be in the minority (perhaps <5 per cent), but could exert a greater influence on the avidity of the antiserum than the lower affinity molecules. Biphasic reaction curves (e.g. MANDEL, 77) may exemplify such mixtures. As the high affinity molecules are diluted, the avidity of the antiserum is reduced as it reflects the lower affinity of the remaining molecules of the majority population.

iii) The Influence of High Affinity Antibody on Low Affinity Antibody

A simplified diagram of how high affinity and low affinity antibodies might interact in an antiserum is shown in Fig. 7. For ease of explanation, all reactive antibodies are represented in the figure as either a single low affinity or a single high affinity molecule. Some low affinity antibody would rapidly react with the antigen but be displaced by the higher affinity (higher

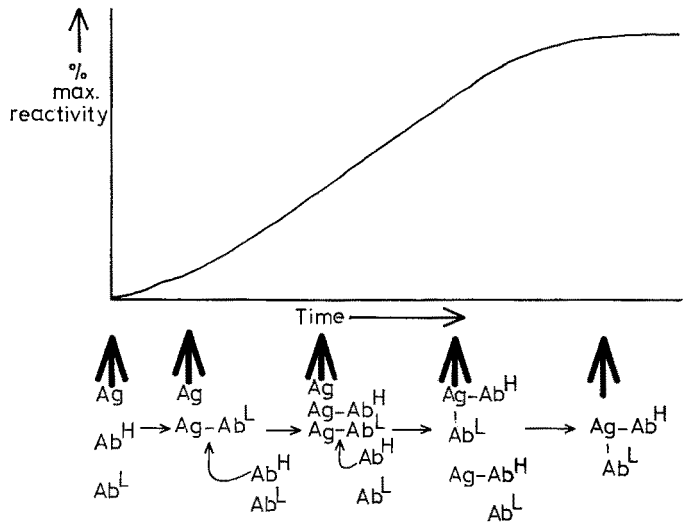

Fig. 7. Representative interaction between antibody molecules in an antiserum which contains only two populations, one of high affinity (Ab^H) and one of low affinity (Ab^L). The graph represents a reactivity curve of antibody with antigen over time

binding constant — stronger binding) antibody. Free antigen and free antibody would continue this displacement phenomenon with high affinity antibody eventually being bound preferentially to the antigen. This high affinity Ag/Ab interaction could however influence the binding of the lower affinity antibody, rendering the binding of the latter stronger than if that low affinity antibody was used alone. Under the appropriate conditions, complexes of antigen with high affinity antibody and low affinity antibody would be found at equilibrium. The situation in an antiserum is of course more complicated, but the figure demonstrates how a minor population of high affinity antibody could influence the reaction by displacing low affinity antibody, and subsequently enhance the binding of that low affinity molecule. Thus, the avidity of a population of antibodies may be unrepresentative of the relative molarities of the constituent high and low affinity molecules (Stanley *et al.*, 113).

The affinities of the antibodies in antisera also vary with time after immunisation (Ozaki, 99). Thus, the proportion of MAb with a particular

affinity derived from each fusion is likely to vary also. If derived from an animal producing an antiserum of avidity influenced as shown in Fig. 7, the majority would be of lower affinity than that of the antiserum. In fact, all MAb produced from a particular fusion may have a lower affinity than the autologous antiserum, if the avidity of that antiserum reflected compensatory or synergistic activities of the constituent antibodies (see above). Many functional activities of MAb against viruses or virus-infected cells may be related to affinity or rely on synergistic reactions. Thus, the failure of a MAb to effect neutralisation of or passive protection against a virus or virus-infected cells does not necessarily indicate that this MAb is ineffective in the immune defence against the virus (see BRUCK *et al.*, 14; LUBECK and GERHARD, 75; CLEGG *et al.*, 20). HEINZ *et al.* (42) demonstrated that mixtures of MAb against tick-borne encephalitis virus gave higher avidities of reaction compared with the individual antibodies. Consequently, the characteristics of some MAb may be irrelevant to the natural polyclonal environment of antisera, particularly when higher affinity molecules influence the reaction of lower affinity antibodies. Affinity and synergistic reactions must therefore be taken into account when attempting to interpret data on the reactivity of MAb.

iv) Specificity of MAb and Autologous Antibody

As with affinity, the specificity and cross-reactivity of MAb may be higher or lower than the autologous antiserum. Interference between antibody molecules could result in an antiserum showing lower cross-reactivity than MAb. The MAb, however, more accurately determine where and when related epitopes (epitypes) exist, not only amongst viruses, but also between viruses, cells and other proteins. Not all MAb necessarily show high degrees of cross-reactivity, and MAb have been used successfully to differentiate isolates amongst many virus groups (for example, OXFORD, 98; YEWDELL and GERHARD, 127; YOLKEN, 128; CROWTHER, McCULLOUGH and CARPENTER, unpublished data). The cross-reactivity and hence the specificity of an antibody or MAb for a determinant on an antigen is due to the paratope of the antibody reacting with that antigenic determinant (epitope). The paratope is the antigen-combining site (crevice) formed between the folded V_H and V_L domains — "variable" regions of the heavy and light chains respectively — of the antibody. Fig. 8 shows a two-dimensional representation of the three-dimensional paratope-epitope interaction. The variations in the size of the paratope are also shown, and the shaded areas in the epitope represent the regions of binding with the paratope. This binding is effected by a combination of electrostatic, hydrogen-bonding and Van der Waal interactions. Each of these individual bonds is weak relative to the overall binding constant, which is due to the interaction of these different bonds. The more bonds involved, the stronger the overall binding (higher

affinity). However, in certain instances, some bonding can compensate for the absence of others, creating an affinity of MAb for a related epitope that is higher than expected.

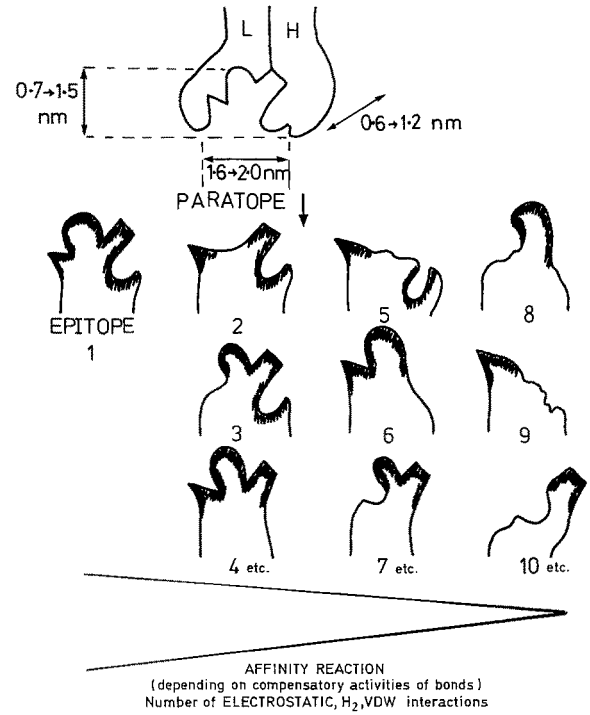

Fig. 8. A two-dimensional schematic representation of three-dimensional interactions between the antibody paratope and reactive epitopes. The shaded area on the epitopes shows where that epitope is likely to form bonds with the paratope

v) Affinity, Specificity and the Paratope-Epitope Interaction

Considering the epitope patterns in Fig. 8, the paratope would have the highest affinity (strongest binding) for the epitope 1, and lowest for epitopes 8 to 10. However, the probability of finding complementary sequences of conformations between two epitopes is higher when using antibody raised against epitope 1 than when using antibody raised against, for example, epitope 8. Anti-epitope 1 MAb would cross-react with ten epitopes (1 to 10, Fig. 8), whereas anti-epitope 8 MAb would cross-react with six epitopes (1, 3, 4, 6, 7 and 8, Fig. 8). Conversely, an anti-epitope 1 MAb would have high affinity for only epitope 1, with decreasing order of affinity (strength of binding) as shown in Fig. 8. Anti-epitope 8 MAb, on the other hand, would have similar affinities for all the epitopes 1, 3, 4, 6, 7 and 8 (providing the conformation of the other epitopes did not sterically interfere with the binding of anti-epitope 8 MAb). Thus, considering all reactions irrespective

of affinity, anti-epitope 1 MAb would have a higher degree of cross-reactivity than anti-epitope 8 MAb. On the other hand, considering only affinities which are similar to those of the homologous reaction, anti-epitope 8 MAb has higher cross-reactivity than anti-epitope 1 MAb. These considerations are important when MAb are used in diagnostic and serological differentiation tests, since tests can be designed to compensate for low affinity reactions.

vi) Other Differences in the Properties of MAb and Antisera

MAb can differ from the autologous antiserum in other immunochemical properties which influence the utilisation of MAb in studies on antibody affinity and specificity. An antiserum may immunoprecipitate a virus or virion protein, but not all constituent antibody molecules (and hence derived MAb) may be capable of this (e.g. see IMAI *et al.*, 49). Similarly, not all antibody isotypes will efficiently fix complement. These properties are relevant to virus diagnosis since immunoprecipitation tests, such as immuno-electrophoresis and Ouctherlony double diffusion, and complement-fixation tests are still widely used as diagnostic assays. In addition, polyclonal antisera may give misleading results. For example, LACHMANN *et al.* (68), using MAb against human C3 antigen, and CROWTHER and McCULLOUGH (unpublished data) with MAb against FMDV have shown that some MAb can still react with an antigen which was apparently saturated by specific antiserum.

The use of different assay procedures with the same antibody and/or antigen can also give conflicting results. If only 50 per cent of the antigen-reactive antibody molecules in an antiserum react in one test, whereas 100 per cent react in a second test, the difference in titre will be two-fold, which is difficult to identify confidently because of variability in the test system. However, only half of the possible antigen-reactive MAb would be detected by the first test, and hence the remaining MAb would have been incorrectly identified as non-antigen reactive. A greater loss in sensitivity of detection occurs when two tests show a 10-fold (one l_{10}) difference in titre. Only 10 per cent of the antibody molecules would react in one test compared with the other, and again only one of the two tests would correctly identify all of the antigen-reactive MAb present. Comparing neutralisation and ELISA titres of antisera autologous to anti-FMDV MAb, differences of 50- to 500-fold in favour of the ELISA are found (McCULLOUGH, CROWTHER, unpublished data). This is not surprising since virus neutralisation tests detect a specific sub-population of antibody, whereas ELISA is less discriminatory. However, variations in the procedure and reagents of an ELISA can also alter the reactivity, and thus detection, of MAb (81, 82). Finally, complement-mediated or cell-mediated lysis of virus infected cells with which MAb have reacted is also variable. The success of these tests in

detecting MAb rely on three major criteria. Firstly, the MAb must be of the correct isotype and density on the infected cell to fix complement. Secondly, it must react with epitopes close enough to the cell membrane to provide rapid attachment of activated $C4_b$ and $C3_b$. Thirdly, these epitopes must be of the correct density such that the cell cannot repair the complement-mediated or leucocyte-mediated membrane damage and hence undergo cytolysis.

vii) Preparation of the MAb

The final consideration for the utilisation of a MAb in the study of epitope relationships is the method of preparing the MAb. The use of concentrated hybridoma supernatants as opposed to ascitic fluids guarantees that the MAb is the only mouse protein present, and can therefore be more easily purified. Cross-reacting protein can be found in ascitic fluids, and natural antibodies reactive against the antigen in question may also be present in variable quantities. Determination of the MAb concentration is also more difficult with ascitic fluids and this is a major problem since antibody concentration is required for kinetic studies and measurements of affinity.

viii) Affinity and Specificity of MAb — Concluding Remarks

In conclusion, both the affinity and specificity of MAb for a particular epitope can differ considerably from autologous antiserum. Other properties of antibody, use of which is made in conventional diagnostic assays, may also differ between the MAb and antiserum. However, careful preparation of the MAb and accurate estimation of its concentration yields a product which can be used to determine the relevance of affinity to certain antibody-mediated immunological functions against viruses, the degree of relatedness between different epitopes both on viruses and between viruses and other biological material, the relatedness of virus isolates and strains in which affinity of reaction may be the conclusive test, and diagnosis of a virus or a particular subtype of virus, which again may rely on affinity measurements. Unlike antisera which contain antibodies of different affinities, the single affinity of a MAb is more readily determined (110), and can therefore be used for rapid diagnosis.

The ability of MAb both to react with the antigenic determinants (epitopes) of a virus and to effect alterations in or protection against the virus (neutralisation) is dependent on the affinity of the antibody. So far, this review has concentrated on discussing these areas of antibody/virus reaction. There is another area of immunology which is relevant to virology — namely idiotopes and idiotope/anti-idiotope interaction. Idiotopes are determinants which confer selfness or uniqueness to an antibody molecule or a family of closely related antibody molecules.

Idiotopes and Idiotope/anti-Idiotope Interactions

A virus is recognised by a host's immune system as foreign due to the antigenic determinants or epitopes on its surface. An antibody molecule also carries "foreign" antigenic determinants. These are classified as xenotypic, isotypic, allotypic and idiotypic. Xenotypic determinants distinguish species of animals. For example, a rabbit responds against xenotypic determinants on Balb/c mouse antibody, whereas a C57/Bl6 mouse does not (the rabbit may also respond against the other classes of determinant). Isotypic determinants are a form of xenotypic determinant which subdivide immunoglobulins into classes and subclasses. Allotypes distinguish strains within a species. For example, C57/Bl6 mice respond to these as well as to idiotypic determinants on Balb/c mouse antibody. The idiotypes distinguish different antibody molecules or families of closely related antibody molecules with a particular antigen specificity. A Balb/c mouse responds to the idiotypic determinants on syngeneic (from other Balb/c mice) or autologous (from the same Balb/c mouse) antibody under appropriate conditions (see below). This response is the basis of the network theory of immune regulation proposed by JERNE (53). The idiotypic determinants can be subdivided into private idiotopes, or cross-reactive and public idiotypes. The term idiotope is used to describe that determinant on a single clone of antibody which confers "selfness" to that antibody. Idiotypes are families of closely related idiotopes, or idiotopes common to a limited number of antibody clones which often have closely related antigenic specificity.

The area of idiotopy which is of direct relevance to virology is that concerned with the "surrogate antigen", and the anti-idiotype antibody which is an internal image of a virus epitope. That is, the anti-idiotype antibody which was induced by the paratope of a virus-specific antibody molecule. From the theory of the surrogate antigen comes the proposal for anti-idiotype vaccines, and this area has been recently reviewed elsewhere (86).

i) Induction of Anti-Idiotype Antibody

When mice are passively immunised with MAb, the anti-idiotype networks (53) may be stimulated, depending on the topographical position of the inducing idiotype on the antibody molecule. Stimulation of an immune response against idiotopes and idiotypes (Id) can only occur when the Id reach immunogenic levels; that is, when the antibodies carrying them are produced in large quantities such as during an active immune response or after inoculation of immunogenic doses (usually 100 μg for small animals). Id can be found within or outside the paratope (antigen-binding crevice) of the antibody, but always within the variable region F_V (10). The variable (V) domains of an antibody are composed of hypervariable

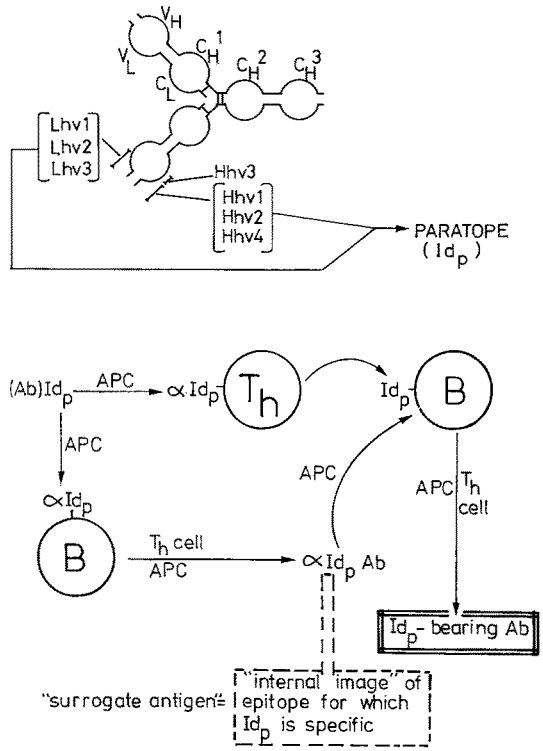

Fig. 9. Idiotype/anti-idiotype interactions associated with the idiotypic region(s) of the inducing antibody paratope (Id_p) and the production of the "internal image" antibody of the "surrogate antigen". For explanation, see text

(hv) regions within the relatively more conserved framework regions. The folding of the heavy and light chains brings most of these hv regions within close promixity of one another into the walls of the antigen-binding crevice (paratope) (10, 44). On the light chains of the variable region (V_L) are the Lhv1, Lhv2 and Lhv3 (Fig. 9), although Lhv2 is occasionally absent from the antigen-binding crevice, being located elsewhere in the V_L domain. On the heavy chain (V_H), Hhv1, Hhv2 and Hhv4 (Fig. 9) are associated with the paratope, while Hhv3 is outside this antigen-binding crevice (44).

Most of these hv regions could function as Id. Conformational associations of hv regions with one another (in the paratope) or with the framework regions could likewise function as Id. Bona (10) has reviewed the evidence demonstrating Id associated with the combining site of antibody, and other Id associated with the framework segments. Furthermore, when antibody reacts with antigen, hv regions in combination with determinants on the antigen may function as idiotypes (44). Anti-Id antibody has been most efficiently induced by using associated heavy and light chains, although isolated chains have been used occasionally; Id have also been identified

which are unique to heavy chains, light chains or both chains in association (8, 87, 105).

KENNEDY *et al.* (60) and KENNEDY and DREESMAN (57) have demonstrated a "common" Id on anti-hepatitis B antibody from two individuals which is apparently associated with the paratope of the anti-hepatitis B antibody, since homologous virus antigen inhibited the Id/anti-Id reaction. KENNEDY *et al.* (57) also reported that some, but not all, anti-HSV2 MAb against *distinct* antigenic determinants share an Id in their antigen combining site, which was also found in Balb/c mouse anti-HSV2 antiserum. LIU *et al.* (74), detected "private" Id, cross-reactive Id (CRI), and "public" Id on monoclonal antiinfluenza virus antibody.

ii) The "Internal Image" Anti-Idiotope

Considering the Id associated with the antigen-binding crevice or paratope (Id_p), the flow scheme in Fig. 9 shows how Id/anti-Id interactions can result in the production of antibody against that paratope Id_p by two independent pathways (86). When the Id_p reaches immunogenic levels, anti-Id_p bearing B cells can be stimulated. These lymphocytes, in the presence of antigen-presenting cells (APC) and the appropriate T helper (T_H) lymphocytes, differentiate and produce anti-Id_p antibody. This anti-Id_p antibody is a mirror image of the inducing paratope. Hence, the paratope of the anti-Id_p antibody should be similar conformationally to the epitope with which the Id_p bearing antibody combines. Thus, it is called an "internal image" of the epitope. This internal image antibody is central to the theory of the "surrogate antigen", which is proposed to function as an "immunogen" stimulating Id_p-bearing B cells to differentiate and produce Id_p-bearing antibody again.

The second route by which the Id_p-bearing B lymphocytes can be stimulated requires the inducing Id_p to be presented (by APC) to anti-Id_p bearing T_H lymphocytes. These T_H cells can in turn trigger the differentiation of Id_p-bearing B cells through Id/anti-Id reaction. This route does not directly utilize an internal image antibody or surrogate antigen, and for the purposes of this discussion will not be referred to. If a surrogate antigen can be prepared its prophylactic use may be substantial.

iii) The Surrogate Antigen

Much evidence has been provided for the induction of Id/anti-Id networks using either MOPC proteins or antibody of known specificity (reviewed by EICHMANN, 28; CAZENAVE *et al.*, 19; DAVIE, 26; KELSOE *et al.*, 55; KENNEDY and DREESMAN, 59; LEGRAIN *et al.*, 72; JANEWAY, 52; NISONOFF and GREEN, 91; URBAIN *et al.*, 120). In order to understand further the influence of these networks on the protective immune response against pathogens, anti-Id

molecules must be stimulated by antibodies which are themselves protective by passive immunisation (86). To this end, SACKS *et al.* (102) have protected mice using anti-Id antisera raised against neutralising (by passive protection) monoclonal anti-trypanosome antibody, although this anti-Id antibody preparation did not contain a true internal image because the immunity induced by it was genetically restricted. REAGAN *et al.* (102) have used rabbit anti-Id antibody against the Id of anti-rabies virus glycoprotein MAb to induce virus neutralising antibody, while GHEUENS *et al.* (37) used anti-Id antibody to inhibit virus neutralisation by anti-measles virus MAb. KENNEDY and DREESMAN (58) showed that anti-Id antibody could induce or enhance the production of anti-hepatitis B antibody which expressed an Id common to convalescent anti-hepatitis B antiserum. Attempts to produce monoclonal anti-Id antibody which can function as surrogate antigen have not been as successful (UYTDEHAAG, personal communication; MCCULLOUGH, unpublished data), although FORSTROM *et al.* (33) used what may be an anti-Id MAb to induce delayed type hypersensitivity against the mouse sarcoma MCA-1490. UYTDEHAAG and OSTERHAUS (121) have recently reported on a syngeneic monoclonal anti-Id antibody against the Id_p of anti-poliovirus type II antibody (a CRI). Inoculation of Balb/c mice with the anti-Id preparation induced Id-bearing antibody, but with considerable variation between animals. This induced antibody had a low neutralization titre (100-fold lower than that induced by passive immunisation with the Id-bearing antibody), but could not protect against lethal poliovirus type II challenge. In addition, the occurrence of such an anti-Id antibody was apparently a rare event, being found only once in three independent fusions. These results would suggest that something more than this single anti-Id antibody is required for an effective surrogate antigen; for example, higher affinity antibody, or antibodies of different specificites may be required (see below and ref. 86).

However, the immunostimulatory capacity of anti-Id antibody is concentration-dependent (55, 90). Anti-Id antibody at 10 to 100 ng enhanced the expression of Id-bearing antibody, whereas 10 μg suppressed the response and 1 μg had no observable effect. A population of anti-Id antibodies also has various affinities for the inducing Id. In addition, while some anti-Id antibodies have a paratope which is the mirror image of the complete Id, other "anti-Id" antibodies react with only a portion of the inducing Id.

Theoretically, high affinity anti-Id_p antibody should be the most efficient surrogate antigen (86). Other anti-Id specificities (against Id outside the combining site and against combinations of the antigen-combining site with the antigen), should, in theory, be incapable of inducing the production of Id_p antibody as shown in Fig. 9, unless the induced Id is genetically linked to Id_p. The anti-Id antibody which recognises only a portion of the Id_p and low affinity anti-Id_p antibody may also be incapable of, or inefficient at,

inducing Id_p antibody. REAGAN *et al.* (102) demonstrated that three of five anti-Id antibody preparations (prepared against anti-rabies virus glycoprotein MAb) were against Id in the combining site of the MAb, but only two of these could induce virus neutralising antibody after passive immunisation. This does not exclude a role or requirement for antibody against other idiotypes/allotypes. It is unclear whether a single anti-Id, several different (or related) anti-Id, mixtures of antibody against private Id, CRI and public Id, combinations of anti-Id with anti-allotype (some allotypes and idiotypes are genetically linked — BONA, 10), or other combinations are required for an effective surrogate antigen preparation. LIU *et al.* (74) have demonstrated that some Id on anti-influenza virus haemagglutinin antibodies are found in both the primary and secondary responses, whereas others are expressed only during the primary *or* the secondary response. An additional complication was shown by KENNEDY *et al.* (56), who reported that immunization with certain anti-Id antibodies could result in an *increase* in the pathogenesis of subsequently inoculated HSV2. (These results may demonstrate the capacity of certain anti-Id at the appropriate concentration to induce immunological suppression or contra-suppression — see BONA, 10). Antibody against the receptors of the T_H cells which are essential for the differentiation of the Id_p-bearing B cells may also be important since immunostimulation by anti-Id antibody is apparently directed by T lymphocytes (BONA 10). A monoclonal anti-Id antibody against a Sendai virus-specific T_H cell clone induced both a B and T cell response against the virus (31) which, in contrast to the monoclonal anti-Id preparation against anti-poliovirus antibody (see above, ref. 121), protected mice against a lethal Sendai virus challenge. Certain anti-Id antibody may suppress major Id and permit the expression of silent Id (reviewed by BONA, 10). Therefore, the relevance of the different anti-Id, anti-allotype, etc., antibodies requires greater insight into how the surrogate antigen works and a better understanding of which lymphocytes are stimulated and how they interact.

Conclusion

Certain implications of MAb in virology have been discussed in this article. Firstly, the valency of the antibody, which depends on the source of myeloma cell used in the fusion, can be used to study antibody activity and function. For example, does the function of a MAb "specific" for a particular epitope rely on a bivalent reaction (bridging antigenic determinants or aggregating virus particles) reaction; how does valency influence the kinetics and affinity of MAb reaction?

Secondly, different mechanisms of virus neutralisation can be identified using different MAb, as can viral epitopes which are important for virus neutralisation *in vitro* and *in vivo*, that is, related to the infectivity and

pathogenicity of the virus. MAb may also be used to determine the relative importance and efficiency of different forms of virus neutralisation (or mechanisms for reducing virus titre) both *in vitro* and *in vivo*, and the relationship between *in vitro* virus "neutralisation" and *in vivo* protection.

The topography of neutralisable and non-neutralisable epitopes on virions has been determined using MAb (reviewed by CARTER and TER MEULEN, 18, and POLLOCK *et al.*, 101). Isolating MAb-resistant variants of viruses has provided considerable information on virus infectivity and pathogenicity (18), and how a virus may behave in an immune host.

The *in vivo* effects of MAb can also provide information on the role and relevance of different immunological defence mechanisms against a particular virus or viral epitope, in partiuclar on opsonisation, complement-mediated cytotoxicity, cell-mediated cytotoxicity (ADCC, complement-enhanced ADCC, CTL activity), and combinations of these.

Any particular anti-virus MAb may show differences in both affinity and specificity compared with the autologous antiserum. Despite this, MAb can be and are used for the diagnosis of virus infections (reviewed by OXFORD, 98; YELTON and SCHARFF, 126; YOLKEN, 128) and differentiation of virus isolates (reviewed also by YEWDELL and GERHARD, 127). The greatest success in the latter field has come with viruses such as rabies virus and measles virus which were differentiated inadequately by conventional serological techniques. The relatedness of epitopes and the affinity of MAb for different epitopes or epitypes can also be applied in epidemiological investigations and have been extensively used with influenza viruses. Such studies may also provide information about apparent epitype relationships between viruses and cells.

Finally, MAb have been used successfully for the induction of anti-Id antibody. Anti-Id antibodies have given considerable insight into the immune regulatory networks, and both MAb and anti-Id (anti-MAb) antibody may have potential as prophylactic agents. However, induction of the anti-Id prophylactic agent pertinent to virology — the surrogate antigen — requires a greater understanding of this branch of immunology. If the anti-Id antibody does show prophylactic potential (as demonstrated by SACKS *et al.*, 107), it may prove useful in vaccine research, although the host range with which a mouse or rabbit anti-Id antibody is effective requires more detailed studies. [URBAIN *et al.* (120) reported that certain anti-Id antibodies against anti-tobacco mosaic virus (TMV) antibody recognised Id on anti-TMV antibody from a wide range of animal species, and could induce anti-TMV antibody; perhaps a species non-specific surrogate antigen]. Particular anti-Id (anti-MAb) antibodies have potential in other areas of research. The internal image anti-Id (against the MAb paratope) may be used to study a viral epitope in isolation, something which is difficult to achieve biochemically due to the denaturation of conformations after

chemical degradation procedures. Monoclonal anti-Id antibodies against anti-reovirus type 3 haemagglutinin antibody bind to receptors for this virus on lymphoid and neural cells (54), enabling a study of virus/cell interactions at the level of single molecules.

There are other uses to which MAb have been or could be put in virology. Some are preparatory, such as immunadsorbent columns, while others are investigative. One topic in this latter area which has received little attention using MAb concerns antibody-induced membrane and cytoplasmic changes in virus-infected cells (reviewed by OLDSTONE *et al.*, 96). The outcome of such antibody pressure may be to maintain a chronic or persistent infection, as has been proposed for latent infections of man such as subacute sclerosing panencephalitis — FUJINAMI and OLDSTONE (35) — rather than directly or indirectly causing destruction of the infected cells. Most of the work in this field has been done using polyclonal antibody, but such "modulation" of virus antigens could be studied at the single epitope level using MAb. This is most important since GLENNIE and STEVENSON (38) reported that monovalent antibody was more efficient than bivalent antibody at destroying L2C leukemia cells since the former avoided antigenic modulation. Recently, FUJINAMI *et al.* (34) used MAb to study antigenic modulation in measles virus infected cells, demonstrating that MAb against the haemagglutinin altered the expression of other virion polypeptides.

All in all, MAb provide information about virus structure and the immunological defence mechanisms against virus infection which could not otherwise be obtained. There are certainly idiosyncracies with MAb, and the hybridoma technology used to produce them. The use of a homogeneous population of a single clone of antibody is totally artificial. The natural situation employs various antibody specificities often working in combination. It should therefore be no surprise if particular MAb behave differently to what was expected from studies using antisera. Nevertheless, provided results obtained with MAb are interpreted in the light of all these factors, MAb will continue to expand our understanding of the structure and behaviour of viruses, and the immune responses against them.

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