14 Neutralization In Vivo

It is widely believed that antibody is important in prevention of reinfection and possibly in recovery from infection as well. However, it is far from easy to determine how such antibody acts. However, immunoglobulin-mediated immunity is easily demonstrated by adoptive transfer to naive animals. In this way it was shown that some monoclonal IgGs which neutralize in vitro confer protection in vivo and others do not; the latter include antibodies to mouse hepatitis virus TALBOT et al. 1984; BUCHMEIER et al. 1984, HSV-1 (RECTOR et al. 1982, KÜMEL et al. 1985), HSV-2 (BALACHANDRAN et al. 1982) and bovine coronavirus (DEREGT et al. 1989). Table 7 appears to show a fairly close correlation between these two parameters but this may be the result of a bias against reporting negative data in the literature; certainly it could never be assumed that a neutralizing antibody would protect in vivo. What properties make for protection are not known. There may be a huge range in efficacy: MATHEWS and ROEHRIG (1984) found that one neutralizing mab to Saint Louis encephalitis virus (SLEV) was 1000-fold more protective than other neutralizing mabs to the same protein. They concluded in an earlier study that avidity and topography of binding were important factors in protection by neutralizing mabs to Venezuelan encephalomyelitis virus (VEEV) (MATHEWS and ROEHRIG 1982). Administration of particular neutralizing mabs may prevent death but result in chronic disease (MHV-4, BUCHMEIER et al. 1984; Aleutian disease parvovirus, ALEXANDERSEN et al. 1989). The elimination of the immunoglobulin response in chickens by bursectomy led to the suggestion that endogenously produced antibody potentiates disease during infection by the infectious laryngotracheitis herpesvirus, possibly by increasing the viscosity of the tracheal exudate, leading to death by axphyxiation (FAHEY and YORK 1990). Bursectomy also exacerbated avian influenza (PORTNOY et al. 1973). The beneficial effects of adoptive transfer of immune serum to influenza virus-infected mice was first demonstrated by LOOSLI et al. (1953) and anti-HA is the most effective anti-viral antibody (VIRELIZIER 1975; VIRELIZIER et al. 1976; MCLAIN and DIMMOCK 1989). Inoculation of athymic mice with a type A influenza virus causes a persistent infection. Adoptive transfer of mab to the HA reduced shedding of virus and allowed resquamation of the trachel epithelium to occur, but the virus was not cleared and, as the antibody titre waned, re-desquamation took place (KRIS et al. 1988).

The complexity of neutralization in vivo (protection) was demonstrated by KÜMEL et al. (1985), who adoptively transferred complement-dependent

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Table 7. Correlation between neutralization in vitro and protection in vivo

Family	Virus/protein	Ig[a]	Neutralization in vitro	Protection in vivo	References
Arena	LCMV/GP1	m	+	+	Wright and Buchmeier 1991;
		m	−	+	Baldridge and Buchmeier 1992
Corona	MHV-4/E2	m	+	−	Buchmeier et al. 1984
		m	+	+[b]	
		m	+	+	Talbot et al. 1984
		m	+	−	
	Bovine coronavirus/E2, E3	m	+	+/−[c]	Deregt et al. 1989
	LDV	m	−	+	Harty and Plagemann 1990
Flavi	Yellow fever virus/54K	m	+	+	Gould 1986
	/48K	m	−	+	
	SLEV/E1c	m	+	+	Mathews and Roehrig 1984
		m	+/−	(+)	
Hepadna	Hepatitis B virus/S	m	0	+	Iwarson et al. 1985
Herpes	HSV-1	m	+	+	Dix et al. 1981
		m	−	+	Balachandran et al. 1982
		m	+/−	+/−	Kumel et al. 1985 Rector et al. 1982; Lausch et al. 1990; Staats et al. 1991
Myxo	Influenza A virus/NA	s	+	+	Schulman et al. 1968
	/HA	s	+	+	Virelizier 1975; Virelizier et al. 1976
	/HA	m	+	+	McLain and Dimmock 1989
	Influenza C virus/HE	m	+	+	Takiguchi et al. 1992
Paramyxo	Murine CMV	m	+/−	+	Farrell and Shellam 1991
	Measles virus/H	m	+	+	Giraudon and Wild 1985
	/F	m	+	+	Malvoisin and Wild 1990
	Simian virus 5/F	m	+	+	Paterson et al. 1987
	RSV/G,F	m	−[f]/+	+	Walsh et al. 1984
	RSV	s	+	+	Prince et al. 1987
		s	+	+	Prince et al. 1990

	RSV/G,F	m	+	+	TAYLOR et al. 1984
		m	−	+	
	PIV-3/F,HN	m	+	+[e]	RYDBECK et al. 1988
	NDV/HN	m	+	(+)	UMINO et al. 1990b
	/F	m	+	+	
	Mumps virus/F	m	−	+	LOVE et al. 1986
	Sendai virus/HN	m/IgA	+	+	MAZANEC et al. 1987
		m	+	+	PIGA et al. 1990
Parvo	Aleutian disease virus	m	?	+[b]	ALEXANDERSEN et al. 1989
Picorna	FMDV	m	+	+	MCCULLOUGH et al. 1992
		s	+	+	BOLWELL et al. 1992
	TMEV	m	+	+	FUJINAMI et al. 1989
Reo	Rotavirus/VP4	m	+/−	+/−	OFFIT et al. 1986
	/VP7	m	+	+	MATSUI et al. 1988, 1989
	Bluetongue virus	m	+	+	LETCHWORTH and APPLETON 1983
	Reovirus/σ1	m	+	+	VIRGIN et al. 1988
		m	−	+	
Retro	HIV-1/gp120	m	+	+	EMINI et al. 1990, 1992
Rhabdo	Rabies virus/G	m	+	+	DIETZSCHOLD et al. 1990a
	VSV/G	m	−	+[d]	LEFRANCOIS 1984
		m	+	+	
	Egtved virus	m	+	+	LORENZEN et al. 1990
		m	−	+	
Toga	Sindbis virus	m	+	+	SCHMALJOHN et al. 1983
		m	−	+	
		m	+	+	LEVINE et al. 1991
		m	+	−	
	Semliki forest virus	m	+	+	BOERE et al. 1983, 1984, 1985
		m	−	+	
	Semliki forest virus (peptide)	s	−	+	GROSFELD et al. 1989
		s	−	+	SNIJDERS et al. 1991
	VEEV	m	+	(+)	MATHEWS and ROEHRIG 1982

[a] Immunoglobulin as antiserum (s) or monoclonal antibody (m).
[b] Against death, but chronic disease ensued; a mixture of four mabs was used; no data on neutralization.
[c] Possibly because different strains were used in vitro and in vivo.
[d] Fc region required; only $F(ab)_2$ were used.
[e] None protected individually but a mixture did protect.
[f] Neutralizes only with complement.
0, No in vitro assay available; (+), some mabs give better protection than others; +/−, some mabs neutralize and do not protect, others do not neutralize and do protect.

neutralizing mabs to HSV-1 gB, gC and gD envelope proteins into DBA-2 mice, which are deficient in C5. Some antibodies protected well but there was no correlation with any known parameter; indeed, mabs to different epitopes on the same protein which neutralized (with complement) to the same extent in vitro protected to different extents. Since the mice are complement-deficient, KÜMEL et al. (1985) suggest that protection is mediated by a mechanism other than neutralization of virions, but this ignores the interaction of virus–mab complexes with C1, C2, C4 and C3. Non-neutralizing mabs similarly protected C5-deficient AJ mice from a lethal dose of HSV-2 (BALACHANDRAN et al. 1982).

The mechanism of protection by antibody may also be complex. LEVINE et al. (1991) found that the adoptive transfer of certain neutralizing mabs to just two epitopes of the E2 protein of Sindbis virus clears virus from the central nervous system of persistently infected mice. A pulse of mab given at 2 days post-infection and removed after 4 days led to the clearance of infection even though large amounts of infectious virus were still being produced at the time antibody was removed. Intracellular markers of infection (viral plus strand RNA synthesis and cytopathic vesicles) were eliminated or reduced. The conclusion that antibody acted by affecting transcription or translation rather than by neutralizing virus per se recalls the modulation of the intracellular expression of the measles virus genome by antibody (Sect. 17).

Possible reasons for the inability of certain mabs to protect are many. Amongst them could be:

1. Cell-specific neutralization, as described by KJELLÉN and SCHLESINGER (1959), KJELLÉN and VON ZEIPEL (1984), KJELLÉN (1985), GRADY and KINCH (1985) and PHILPOTT et al. (1989); i.e. antibody neutralizes when infectivity is assayed on cultured cells in vitro but less effectively or not at all with the target cell in vivo (see Sect. 7).
2. Affinity of IgG: if this were too low the antibody might be ineffective in vivo.
3. The ability to activate complement, which in turn depends on the particular immunoglobulin isotype under study.

Antibody may act in vivo not by neutralizing virus infectivity but by interaction with viral antigens on the cell surface, and this can also involve non-neutralizing antibodies to internal virion and non-structural antigens. It is possible but not proven that such antibodies protect in vivo by activation of components of the complement system or by interaction with Fc receptors on cells (mostly of the monocyte/macrophage lineage) which then exert anti-infected cell activity (VEEV, MATHEWS et al. 1985; VSV, LEFRANCOIS 1984; yellow fever virus, GOULD 1986; dengue virues type 2, SCHLESINGER et al. 1987; Semliki forest virus, GROSFELD et al. 1989). There appears to be a varying requirement for intact antibody, rather than $F(ab)_2$, for protection but in most cases there was a requirement for a property other than that of complement activation. Neither neutralizing nor non-neutralizing $F(ab)_2$

protected mice from LCMV, and here C5-deficient animals were used to show that the full complement system was not required (BALDRIDGE and BUCHMEIER 1992); similarly only intact IgG protected mice against VEEV, again by a complement-independent mechanism, as shown by the use of C3- or C5-deficient mice (Mathews et al. 1985); and with FMDV, 10- to 500-fold more $F(ab)_2$ than IgG was required for protection of mice (MCCULLOUGH et al. 1986). $F(ab)_2$ prepared from neutralizing or non-neutralizing monoclonal IgGs to Semliki forest virus protected mice poorly or not at all (Boere et al. 1985). However, $F(ab)_2$ prepared from a pool of human sera protected cotton rats against RSV infection, showing that the Fc region was not required (PRINCE et al. 1990). Neutralizing but not non-neutralizing $F(ab)_2$ protected mice from VSV but there are no data on the involvement of complement (LEFRANCOIS 1984).

In a review of the extensive work on paramyxoviruses, NORRBY (1990) concludes that antibodies to the HN/G envelope proteins give better protection than anti-F, but for RSV the converse holds true. Table 7 gives other references to protection by antibodies against paramyxovirus infections. UMINO et al. (1990b) comment that the best predictor of protection is not the neutralization titre but a high ratio of haemagglutination-inhibition: neutralization titre and/or the ability to inhibit plaque formation when antibody is incorporated into the overlay medium. Recent data on protection against the primate lentiviruses HIV-1 and SIV suggest that antibody mediates protection; this is shown most clearly from the passive transfer of HIV-1 gp120-specific neutralizing mabs into chimpanzees (EMINI et al. 1990, 1992). The, as yet, unresolved confusion concerning the relative contribution of anti-virus and anti-cell antibodies in protection of monkeys against SIV has already been discussed (Sect. 11). Passively transferred immune monkey serum protected cynomolgus monkeys from HIV-2 and SIV_{sm} (PUTKONEN et al. 1991).

Although outside the scope of this review, it should not go unmentioned that both neutralizing and non-neutralizing antibody are thought to enhance the pathogenic potential of some viruses in vivo by permitting virion–antibody complexes to gain entry to cells which they would not normally infect by binding to Fc receptors (see Sect. 8). Suspicions centre on viruses such as dengue (HALSTEAD 1988), rabies (SIKES et al. 1971; BLANCOU et al. 1980; PRABAKHAR and NATHANSON 1981), Japanese encephalitis (GOULD and BUCKLEY 1989), yellow fever (BARRETT and GOULD 1986; GOULD and BUCKLEY 1989) and latterly HIV-1 and SIV (TAKEDA et al. 1988, 1990; HOMSY et al. 1989, 1990; MONTEFIORI et al. 1990; ROBINSON et al. 1989, 1990a,b, 1991), but others are unconvinced (MORENS and HALSTEAD 1990).

In vivo a narrow range of antibody specificities may be produced to influenza virus and may be responsible for driving antigenic variation (drift) by selecting neutralizing antibody escape mutants (HAAHEIM 1980; NATALI et al. 1981; WANG et al. 1986). Neutralization escape mutants of a number of different viruses have reduced virulence (KÜMEL et al. 1985; ROOS et al.

1989; VAN HOUTEN et al. 1991; JOHNSON et al. 1990; KÖVAMEES et al. 1990; CECILIA and GOULD 1991) or cause a different type of disease (ZURBRIGGEN and FUJINAMI 1989). Escape mutants are found in vivo during infection with HIV-1 (ALBERT et al. 1990; EMINI et al. 1990; NARA et al. 1990; MONTEFIORI et al. 1991) and hepatitis B virus CARMAN et al. 1990).

Conclusion. Some neutralizing antibodies protect very effectively in vivo while others do not. This discrepancy is not understood and may be virus–antibody–target cell dependent, as it is in vitro but with the added complexity of the possible involvement of other elements of the defence system.