Single- and multi-hit kinetics of immunoglobulin G neutralization of human immunodeficiency virus type 1 by monoclonal antibodies

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A quantal assay, based on syncytium formation in the human T cell leukaemia-derived C8166 cell line, was used to determine the kinetics of human immunodeficiency virus type 1 (HIV-1) strain IIIB neutralization. Three rat monoclonal antibodies (MAbs) were used, under physiological conditions of temperature and antibody concentration. MAb ICR39.3b (IgG2b) neutralized virus with no lag period while the other two MAbs, ICR39.13g (IgG2b) and ICR41.11 (IgG2a), neutralized with lag periods of 5 min and 15 min respectively. It was calculated that the latter two MAbs mediated neutralization by about two and three molecules of IgG per virion respectively. The highest neutralization rate constant (for MAb ICR 41.11) was over 300-fold less than that of MAbs specific for the haemagglutinin of the enveloped influenza virus type A and for poliovirus type 1.

In general, mechanisms of neutralization of animal viruses are poorly understood. However, it seems clear that the outer protein coat of any one animal virus has multiple copies of several different neutralization sites. These sites stimulate antibodies which vary in paratope and isotype and which neutralize with different efficiencies and probably by different mechanisms (for a review, see Dimmock, 1993). Where there are problems in stimulating protective immune responses, such as with human immunodeficiency virus type 1 (HIV-1), it seems logical to investigate how antibodies neutralize, with a view to preferentially stimulating those antibodies which neutralize most efficiently. Here we have investigated the neutralization kinetics of HIV-1 by three rat monoclonal antibodies (MAbs). Such data are of interest as they can be used to calculate the number of antibody molecules per virion that are required to mediate neutralization and hence give an insight into the mechanism of neutralization of each particular MAb. The data available on kinetics of neutralization are not extensive and have been reviewed by Mandel (1979). Neutralization which proceeds without any lag has been reported with both polyclonal antisera (Dulbecco et al., 1956; Rubin & Franklin, 1957; Mandel, 1960; Thormar, 1963; Granoff, 1965; Philipson, 1966; Keller, 1966; Wohlafort, 1988) and monoclonal IgG (Jenogle et al., 1983; Taylor et al., 1987; Suñé et al., 1990; Outlaw & Dimmock, 1991). This has been interpreted as a single-hit process mediated by one molecule of antibody. For other antibodies a multi-hit mechanism has been proposed, based on the occurrence of a lag period before neutralization com-
linear relationship between the dilution of HIV-1 IIIB and the number of syncytia produced per well over a range of 5 to 50 (Fig. 1). The regression coefficient was 0.996 and the slope was -1.23. At higher concentrations of virus this relationship deviated from linearity, probably because of the formation of secondary syncytia.

For these studies the rat MAbs ICR39.3b (IgG2b) and ICR39.13g (IgG2b), which both recognized conformational determinants on gp120 (Cordell et al., 1991), and ICR41.1i (IgG2a) which recognized a conformational determinant on the V3 loop (McKeating et al., 1992; J. Moore, personal communication) were used. Hybridomas were maintained in Dulbecco's modified Eagle's medium containing 5% FCS and supplemented as for H9 cells. MAbs were purified from tissue culture fluids by affinity chromatography on recombinant Protein G-Sepharose (Gammabind plus; Pharmacia) and protein concentrations were measured by their absorbance (1 A280 unit represents 1.34 mg/ml). The possibility of bovine IgG contaminating the purified MAbs was checked by passing the same volume (200 ml) of fresh medium through Protein G-Sepharose. The protein purified in this way was 2.7% of that purified from medium containing MAbs. It had no neutralizing activity against HIV-1 (data not shown). The MAbs gave 50% neutralization with 5 g/ml of ICR41.1i, 50 g/ml of ICR39.13g and 94 g/ml of ICR39.3b (data not shown).

In order to study the kinetics of neutralization, HIV-1 IIIB was diluted to give about 1.2 x 10^5 syncytium-forming units per ml. Each MAb was diluted to a level that allowed measurement of the reaction rate and mixed in equal volumes with virus to a final concentration of 30 syncytium-forming units/well. The mixture was incubated for various times at 37 °C and then inoculated onto C8166 cell monolayers to assay residual infectivity. After allowing exactly 1 h for incubation of each sample with the cells, the inocula were removed. Fresh medium was then added to each monolayer before incubation for 3 days at 37 °C. Syncytia were counted and the neutralization kinetics determined by regression analysis.

Fig. 2 shows that the three MAbs have different neutralization kinetics. MAb ICR39.3b (Fig. 2a) neutralizes with no initial lag. The rate of neutralization decreased approximately 3.5-fold after incubation for 15 min but remained linear for over 100 min. In contrast,
neutralization with ICR39.13g and ICR41.1i (Fig. 2b and c) showed an initial lag period. Although the lag with ICR39.13g (5 min) is represented by a single data point, it was reproduced in other experiments (data not shown). Furthermore, when compared with the data for ICR39.3b (Fig. 2a), it is apparent that this substantial lag period is not an artefact of the procedure. Neutralization with ICR41.1i showed a lag of 15 min, and this was unequivocally demonstrated with three data points. Such kinetics are characteristic of a multi-hit process which requires the binding of more than one antibody molecule before neutralization can commence. The rate of neutralization of ICR39.13g decreased by about 3.3-fold after 15 min incubation and then remained linear for 75 min. Once it had commenced, neutralization by ICR41.1i gave a constant rate of reduction of infectivity for the duration of the experiment. This decrease in the rate of neutralization seen after 15 min with ICR39.3b and ICR39.13g (Fig. 2a and b) has been observed many times with other viruses but no satisfactory explanation has been suggested. As we are using MAbs here, heterogeneity of the antibodies cannot be the cause. Virus heterogeneity is possible, but since ICR41.1i did not exhibit any change in the rate of neutralization, such variation would have to affect some but not all epitopes.

The rate constant (k) of a neutralization reaction is calculated using \[ \log_{10} \frac{V_t}{V_0} = (-k/2.3D)t, \] where \( V_0 \) and \( V_t \) are the infectivity titres at time 0 and t respectively and D is the molar concentration of the antibody. The greater the value of k, the better the antibody concerned can neutralize. Reaction rate constants (k1 for the initial reaction and k2 for the subsequent slower reaction) were calculated for each MAb from Fig. 2. The lag periods were not included in these calculations. The \( M_f \) for IgG was approximated to 150000. The \( k_1 \) and \( k_2 \) values shown in Fig. 2 demonstrate that the order of neutralization effectiveness for the MAbs is ICR41.1i, ICR39.13g and then ICR39.3b. However, an evaluation of neutralization has to take into account the lag periods of the MAbs.

These anti-HIV-1 k values can be put into context by comparison with other k values. For example, a MAb against influenza virus type A (A/FPV/Rostock/34:H7N1) haemagglutinin was found to have a k value of approximately \( 2 \times 10^8 \) M\(^{-1}\) s\(^{-1}\) at 37°C when assayed on Madin–Darby canine kidney cells. This is over 300-fold greater than ICR41.1i (unpublished results). An anti-poliovirus MAb has been reported to have a k value of approximately \( 4 \times 10^8 \) M\(^{-1}\) s\(^{-1}\) at 20°C (Icenogle et al., 1983).

Kinetic neutralization curves that extrapolate through the origin (Fig. 2a) have been observed for many different viruses and with both polyclonal sera and MAbs. For example, the neutralization of HIV-1 by chimpanzee and human antisera appeared to have this kind of curve, although a detailed analysis was not possible from the data presented (Nara, 1989). There are at least three possible mathematical explanations of such data for IgG (Icenogle et al., 1983). Firstly, if all the epitopes relevant to this MAb mediate neutralization and are therefore critical and infectivity is inactivated with single-hit kinetics, then binding by a virion of a single antibody molecule is responsible for the loss of infectivity. Secondly, if not all the epitopes relevant to this MAb are critical, but infectivity is still inactivated by single-hit kinetics, then a virion will bind more than one molecule of antibody before neutralization commences. Thirdly, there is fractional inactivation of infectivity, whereby each of x antibody molecules binding to a virion causes the loss of \( 1/x \) of the total infectivity of the virion. Again this means that a virion will bind more than one molecule of antibody before detectable neutralization commences. Although the third reaction profile is not linear, the data, such as those presented in Fig. 2(a), are not sufficiently precise to be distinguished from a truly linear curve.

In contrast, the neutralization kinetics seen in Fig. 2(b) and (c), which give an initial lag or shoulder before loss of infectivity commences, have not been reported before with IgG at physiological temperatures and antibody concentrations. They are interpreted as indicating that neutralization proceeds by a multi-hit process, whereby the cooperative binding to a virion of two or more antibody molecules is required before it loses any infectivity. The precise number of hits or antibody molecules (n) required to neutralize each virion can be calculated using the equation \[ V_t/V_0 = 1-(1-e^{-kt/D})^n. \] From this it was calculated that MAb ICR39.13g required 2-3 antibody molecules per virion and MAb ICR41.1i required three molecules per virion for neutralization. The significance of these data to the molecular mechanism of neutralization is currently under investigation and will be reported elsewhere. It is probably coincidental that the most efficient MAb at neutralization, ICR41.1i, acts with multi-hit kinetics. The multi-hit neutralization reported here is unrelated to isotype as ICR39.13g and ICR39.3b, which gave no lag in neutralization, are both IgG2b.

In terms of efficiency of neutralization a MAb like ICR41.1i, which neutralizes with multi-hit kinetics and a lag of 15 min, is intrinsically worse than a MAb that has the same k value and neutralizes with no lag period. This is because prevention of infection is essentially a race between neutralization and the escape of the virus particle from neutralization by entry into a host cell. This indicates that, in vivo, it is preferable to have a vaccine which stimulates antibodies with a high k value and that neutralize with apparently single-hit kinetics.
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References


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