Antibody Synthesis *in Vitro*: The Antigenic Reactivity of Antibodies to Coliphage MS-2 Synthesized in the Presence and Absence of Streptomycin[1] (35135)

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The experiments reported here demonstrate that antibodies synthesized in the presence of 200 μg/ml of streptomycin (Sm-antibodies) have lower avidity (the relative ability to form stable complexes with antigen) and/or affinity (the relative rate of association with antigen) for infectious MS-2 particles than antibodies synthesized in the absence of the drug (N-antibodies). These results provide an explanation as to why Sm-antibodies are relatively inefficient in neutralizing phage MS-2 [Krueger (4, 5)].

Methods. Preparation and purification of phage MS-2, immunication of the rabbits, preparation of spleen and lymph node cell suspensions for *in vitro* culture and isolation of antibodies produced in *in vitro* cultures have been described previously [Krueger (4, 6,)]. Spleen and lymph node cells were cultured *in vitro* in Eagle's minimal essential medium at 37° in an environment of 90% air–10% CO_2 in the presence or absence of 200 μg/ml of Sm and phage MS-2.

Measurements of avidity. For measuring the avidity of the N- and Sm-antibodies, approximately $1–2\times10^5$ PFU of phage MS-2 were added to a dilution of an *in vitro* antibody preparation and the mixture was incubated at 37°. At appropriate time intervals, 0.1-ml samples were removed and diluted with phosphate buffered saline (PBS) at 4° to stop further inactivation of the virus. For the regular titration, aliquots of these dilutions were assayed for viable phage particles by incubation at 37° for 10 min with a broth culture of *Escherichia coli* AB261. The mixture was plated with 2 ml of tryptone soft agar and incubated overnight at 37°. In this method of titration, inactivated phage particles that dissociate from the antibodies during the overnight incubation period appear as unneutralized phage particles that can infect sensitive bacteria and produce plaques.

In order to inhibit dissociation of the phage–antibody complexes, the *in vitro* antibody preparations were titrated by a modified decision-tube titration (Jerne and Avegno (3)]. Samples of the same diluted phage–antibody complexes used for the regular titration assay, were incubated with sensitive *E. coli* AB261 at 37° for 10 min; and 0.1 ml of a diluted hyperimmune antiserum was added to a final concentration of 0.1 K units in the plating mixture. After incubation at 37° for 10 min, 2 ml of tryptone soft agar were added, the mixture was plated and incubated overnight at 37°. In this titration, unneutralized phage can only infect the sensitive bacteria during the initial 10 min incubation period, since the addition of the hyperimmune serum neutralizes any phage that dissociates from the antibody after this period.

As a second measurement of aviditiy, the percentage of the phage reactivated during the overnight incubation was calculated at equivalent points in the plateau region of neutralization curves obtained by both the regular and decision-tube titration methods.

The rate constant of phage neutralization, K, was calculated from the linear portion of the neutralization curve as described by Adams (1). The ratio of the K of the decision-tube titration (reactivation K) to the K of the regular titration (regular K), was em-

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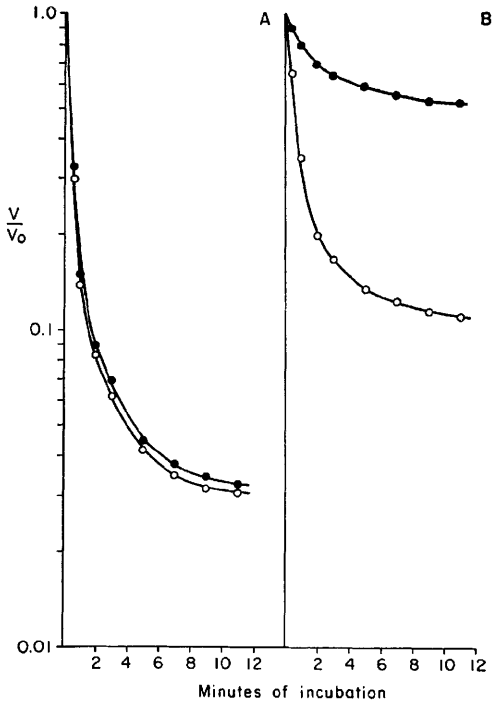

FIG. 1. Avidity of N- and Sm-antibody preparations diluted 1:5 (A) N-antibody; (B) Sm-antibody. Regular titration (●); decision-tube titration (○).

ployed as one measurement of avidity (*K* ratio).

In order to compare the rate at which N- and Sm-antibody preparations reacted with a comparable concentration of infectious MS-2 phage particles, the preparations were diluted to the same K concentration, mixed with 1–2 × 10^8 PFU of MS-2 and incubated for up to 96 hr at 4°. The reaction mixtures were sampled at various time intervals, diluted, and plated by both the regular and decision-tube titration procedures. This reaction, termed a long-term static neutralization reaction, is essentially an equilibrium reaction and measures the rate and maximum extent of phage neutralization by a given concentration of MS-2 antibody.

Results. Figure 1 demonstrates the typical kinetics of phage inactivation and reactivation observed for equivalent dilutions of N- and Sm-antibody preparations produced in parallel cell cultures. The N-antibody preparation neutralized phage MS-2 to a low level and the neutralization curves from the regular and decision-tube titrations closely approximated one another. This indicates the N-antibodies had high avidity for MS-2. However, Sm-antibody preparations demonstrated neutralization kinetics by the regular and decision-tube titration procedures that did not approximate one another. By the regular titration, virus inactivation occurred to a relatively moderate level whereas, by the decision-tube titration, virus inactivation occurred to a level comparable to that observed with N-antibodies. These data indicate that Sm-antibody preparations possess low avidity for phage MS-2. However, it also indicates that these antibody molecules are capable of reacting with the phage antigen.

Table I shows the difference in avidity between representative N- and Sm-antibody preparations produced in parallel cell cultures. N-antibodies demonstrated high avidity that was comparable to three hyperimmune antisera, whereas Sm-antibodies demonstrated lower avidity. However, the avidity differences between N- and Sm-antibody preparations suggest that dissociation of phage–antibody complexes on the assay plates could not entirely account for the differences in neutralizing activity of compar-

TABLE I. Average Avidity of Hyperimmune Serum, N- and Sm-antibodies for MS-2.

Antibody	Regular *K*	Reactivation *K*	*K* Ratio	Phage reactivation (%)
Serum no. 1	33,000	38,300	1.16	1.25
2	23,000	28,800	1.25	1.43
3	12,100	16,500	1.36	3.18
N- no. 1[a]	19.1	23.0	1.21	1.32
2	29.9	31.2	1.04	1.10
3	31.5	34.4	1.09	1.15
4	31.5	41.1	1.31	1.51
Sm- no. 1[b]	0.92	15.0	16.3	15.1
2	3.8	21.5	5.66	6.3
3	0.57	19.3	33.86	37.3
4	0.73	11.5	15.75	14.5

[a] Representative N-antibody preparations synthesized *in vitro* in 1-day-old spleen cell cultures.

[b] Representative Sm-antibody preparations synthesized in 1-day-old spleen cell cultures.

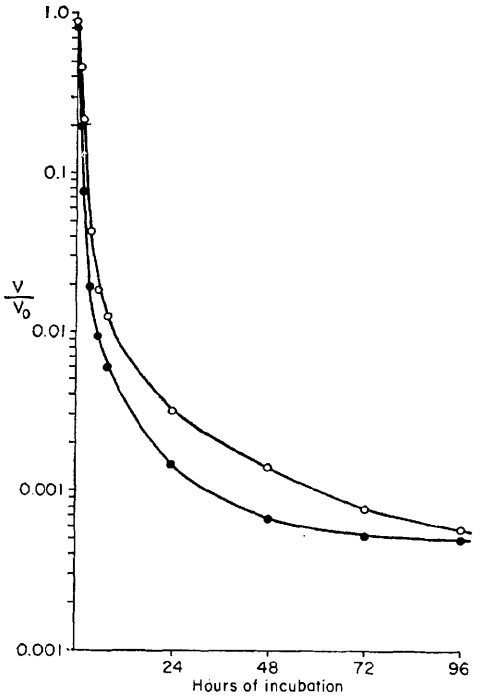

FIG. 2. Neutralization of phage MS-2 by N- and Sm-antibodies in the static neutralization assay. Both antibody preparations were diluted 1:4 and neutralization was determined by the decision-tube assay. N-antibody (●); Sm-antibody (O).

able dilutions of N- and Sm-antibody preparations.

Figure 2 illustrates the binding affinity of equivalent dilutions of N- and Sm-antibody preparations. N-antibodies demonstrated a greater capability to associate with and neutralize MS-2 than did comparable dilutions of Sm-antibodies when assayed by the decision-tube titration procedure. When equivalent antibody concentrations (equivalent K concentrations) of a N- and a Sm-antibody preparation were reacted in a long-term static reaction (Fig. 3), the Sm-antibody preparation demonstrated a different maximum level of phage inactivation in addition to a lower rate of phage inactivation than the N-antibody preparation by both titration procedures. These data indicate that the Sm-antibody preparation, in addition to lower avidity, had a lower rate of association (affinity) with the MS-2 phage antigen than the N-antibody preparation. This difference in avidity and/or affinity between N- and Sm-antibody preparations was routinely observed in 15 parallel antibody preparations synthesized in cell cultures from 7 different rabbits.

Discussion. Sm-antibody preparations by the regular titration procedure demonstrated markedly lower neutralizing activity for phage MS-2 than did equivalent dilutions of N-antibody preparations (Fig. 1).

The disparity between the neutralizing activity of these two antibody preparations as determined by the regular titration, could be explained if (i) Sm-antibodies reacted with the phage particle at a "noncritical" site and so were unable to neutralize its infectivity; (ii) Sm-antibodies possessed low avidity for MS-2 and readily dissociated from the infectious particles; (iii) Sm-antibodies possessed low affinity for MS-2 and neutralized the phage with different kinetics.

The difference between the neutralization data generated by the two titration procedures indicates that Sm-antibodies had a

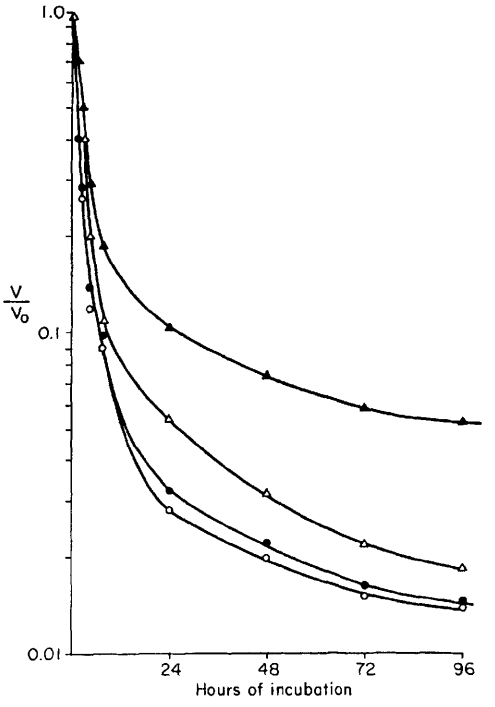

FIG. 3. Neutralization of phage MS-2 by N- and Sm-antibodies in the static neutralization assay. Both antibody preparations were diluted to the same *K* concentration/ml. N-antibody, regular titration (●); decision-tube titration (O); Sm-antibody, regular titration (▲); decision-tube titration (△).

lower avidity for MS-2 phage than did N-antibodies and formed unstable antibody–phage complexes. In addition, comparison of the neutralizing activities of equivalent dilutions or equivalent antibody concentrations of N- and Sm-antibody preparations in long-term static neutralization reactions revealed that Sm-antibodies formed stable complexes with infectious MS-2 particles at a relatively slower rate than did N-antibodies. Sm-antibodies, therefore, had lower affinity for the homologous antigen than did N-antibodies. The difference in avidity and affinity accounts for our previous observations that Sm-antibodies had a lower capability than N-antibodies in neutralizing the infectivity of the homologous phage antigen [Krueger (4, 5)]. Thus, it is evident that by whatever mechanism Sm affects antibody synthesis in lymphoid cells, antibodies synthesized in the presence of relatively high concentrations of the drug are not altered to the extent that they are unable to react with the homologous phage antigen.

Summary. Spleen and lymph node cells from rabbits hyperimmunized with coliphage MS-2 synthesized neutralizing antibodies when cultured *in vitro*. Antibodies synthesized in cell cultures in the absence of streptomycin neutralized phage MS-2 to a high titer whereas antibodies synthesized in the presence of 200 μg/ml of the drug had reduced neutralizing capacities. The difference in neutralizing activity was due to the fact that antibody preparations synthesized in the presence of the drug had either lower avidity and/or affinity for phage MS-2 than did antibody preparations synthesized in the absence of the drug.

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