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# Influence of Complement on the Neutralization of Murine Cytomegalovirus by Rabbit Antibody

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Antiserum against murine cytomegalovirus produced in the rabbit contained complement (C')-requiring neutralizing (CRN) antibody. The proportion of CRN was extremely high (up to 98%) during the early portion of an immunization procedure, whereas the antisera produced late had a much lower proportion that required C'. The antiserum produced was specific for MCMV with or without C'.

Several animal viruses, including herpes simplex type 1 (HSV-1), simian cytomegalovirus, and rubella, are neutralized more efficiently by antibody after addition of complement (C') to the reacting virus-antibody mixtures (1, 6, 8). More recently, Anderson (2) and Graham et al. (3) demonstrated that anti-human cytomegalovirus (HCMV) prepared in rabbits and goats contained a large amount of complement-requiring neutralizing antibody. We studied the effect of fresh C' on neutralization of murine cytomegalovirus (MCMV) with antibody prepared in rabbits. The sera produced early during an immunization protocol were highly dependent upon C', whereas those obtained later were not as dependent.

Mouse embryo fibroblast (MEF) cells and embryonic human fetal tonsil (FT) cells were used for growth and quantitation of MCMV and HCMV strains, respectively, as described (4, 5). HSV-1 was grown and quantitated in FT cells (7). Three established laboratory strains of HCMV, i.e., AD169, Davis, and Espilat, and the Smith strain of MCMV were used.

Rabbits were immunized with virus that had been concentrated 50 times. Virus pools were pelletized by centrifugation at 25,000 rpm for 1 h, and the pellets were washed three times with phosphate-buffered saline. The pellets were then taken up in 0.02 vol (phosphate-buffered saline) of the original pool. At 15 weekly intervals, rabbits were immunized by intramuscular injection. The first five injections were made with Freund complete adjuvant. Rabbits were bled at weekly intervals. This was the same neutralization protocal which Graham et al. (3) have used. Antisera against MCMV were absorbed three times with $5 \times 10^7$ normal mouse embryo fibroblast cells each time to eliminate anti-cell antibody, whereas FT cells were used to absorb antisera against HCMV. Antisera against HSV-1 and HSV-2 were kindly supplied by Berge Hampar of the National Cancer Institute, and they were used without prior treatment.

The effect of different concentrations of C' on neutralization antibody titers of rabbit anti-MCMV was tested. Heat-inactivated C' (56 C for 30 min) and fresh C' were used in parallel to determine the concentrations that would augment neutralization optimally (Table 1). In the presence of heat-inactivated C', the serum titers were 80 or less. This is comparable to the titer obtained in the absence of C'. In the presence of a wide range of active C' concentrations, the serum was 4- to 16-fold higher with C' than

TABLE 1. *Effect of different concentrations of C' on neutralizing antibody titers of rabbit anti-MCMV serum*[a]

| C' tested | | Neutralizing antibody titers[b] | |
|---|---|---|---|
| Dilution | Hemolytic units | Unheated C' | Heated C'[c] |
| 1:1 | 48 | 640 | 160 |
| 1:2 | 24 | 640 | 80 |
| 1:4 | 12 | 640 | 80 |
| 1:8 | 6 | 1,280 | 80 |
| 1:16 | 3 | 1,280 | 80 |
| 1:32 | 1.5 | 640 | 160 |
| 1:64 | 0.75 | 640 | 80 |
| 1:128 | 0.375 | 640 | 80 |
| 1:256 | 0.187 | 160 | 80 |

[a] Serum was absorbed with normal mouse embryo fibroblast cells before the test.

[b] Reciprocal of the highest serum dilution giving 60% plaque reduction.

[c] Heated at 56 C for 30 min.

TABLE 2. *Kinetics of development of C'-requiring neutralizing antibodies to MCMV in immunized rabbits*

| Bleeding at virus inoculation no.[a] | Neutralizing antibody titer[b] | |
|---|---|---|
| | Without C' | With C'[c] |
| 1 | <10 | 10 |
| 2 | 320 | 10,240 |
| 3 | 80 | 5,120 |
| 4 | 80 | 1,280 |
| 5 | 80 | 320 |
| 6 | 80 | 320 |
| 7 | 80 | 640 |
| 8 | 320 | 320 |
| 9 | 160 | 320 |
| 10 | 160 | 320 |
| 13 | 80 | 320 |
| 15 | 80 | 640 |
| Preinoculation serum | <10 | <10 |

[a] Rabbits were inoculated 15 times at weekly intervals. At the inoculations indicated, serum was taken.

[b] Reciprocal of the highest serum dilution giving 60% plaque reduction.

[c] Ten hemolytic units of C' were used.

TABLE 3. *Specificity of C'-requiring neutralizing antibodies*

| Rabbit antibody | Virus | | | | |
|---|---|---|---|---|---|
| | Cytomegalic | | | | Herpes |
| | Mouse | AD169 | Davis | Espilat | Type 1 |
| Anti-MCMV | 640[a] | <10 | <10 | <10 | <10 |
| Anti-AD169 | <10 | 640 | ND[b] | ND | <10 |

[a] Reciprocal of the highest serum dilution giving 60% plaque reduction.

[b] ND, not done.

without, with the optimal concentrations at 1:8 and 1:16.

Rabbits immunized with MCMV were followed for the development of antibody to the virus by testing sera taken from serial bleedings in neutralization tests with and without C'. During the first 4 weeks there was a markedly higher titer in the presence of C' than in its absence (Table 2). In the latter stage of immunization, the sera obtained did not contain as high a proportion of complement-requiring neutralizing antibody as did earlier sera.

The extent of antigenic relatedness among several members of the herpesvirus group were examined by reciprocal neutralization tests in the presence and absence of C'. Antiserum prepared against MCMV did not neutralize HSV-1 or three strains of HCMV (AD169, Davis, and Espilat) (Table 3). In addition, antiserum against AD169 did not neutralize MCMV or HSV-1. In one further experiment, MCMV was not neutralized by antisera against either HSV-1 or HSV-2.

The importance of incorporating C' in neutralization studies of cytomegaloviruses is emphasized in this study. With the murine strain of cytomegalovirus, C' was particularly important with antisera obtained early. However, even with antiserum produced late in an immunization regimen, the antibody titer was usually higher with C' than without it.

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