Interaction of Rheumatoid Factor with Infectious Herpes Simplex Virus–Antibody Complexes

Abstract. *Rheumatoid factor, a human immunoglobulin of the IgM class, failed to attach to herpes simplex virus but did attach to infectious complexes composed of herpes simplex virus and antibody to herpes simplex virus. These newly formed complexes of infectious virus, antiviral antibody, and rheumatoid factor could be neutralized by complement or by antibody to human IgM. The ability of rheumatoid factor to enhance virus neutralization in the presence of complement represents a hitherto unrecognized biological role for rheumatoid factor.*

Under certain conditions the interaction of antiviral antibody with virus results in the formation of infectious virus–antibody (VA) complexes (sensitized virus) (*1*). These complexes have been demonstrated both in vitro and in the circulation of animals chronically infected with a variety of viruses (*1, 2*). In many of these infections, deposition of circulating VA complexes in the kidneys can result in the development of immune-complex type glomerulonephritis (*2*). Recently, we showed that infectious VA complexes could be neutralized by specific antibodies to immunoglobulins or by the sequential attachment of the individual components of complement (*1, 3, 4*). We now report that rheumatoid factor (RF), a human immunoglobulin of the IgM class, also can attach to these infectious VA complexes.

Herpes simplex virus (HSV) (Type 1, strain 11148) was prepared and assayed by the plaque technique on primary rabbit kidney cells (*5*). The IgG fraction of rabbit antiserum to HSV (anti-HSV IgG) was reduced (0.1*M* 2-mercaptoethanol) and alkylated (0.02*M* iodoacetamide) as described earlier (*6*), and the papain-derived Fab I fragments were the same as those used previously (*3*). The IgG fraction of human antiserum to HSV was prepared by chromatography on Sephadex G-200 and was shown to be free of RF. Human and guinea pig serums with no detectable RF or antibody to HSV served as the source of complement. Antiserums against specific immunoglobulins were prepared in goats and rabbits by Hyland Laboratories, Los Angeles, California.

Rheumatoid factor was prepared by chromatographing, on a Sephadex G-200 column, the euglobulin fraction of serums from patients with rheumatoid arthritis (*7*). The IgM peak was concentrated and had an RF titer of 1:1280 as measured by latex agglutination (*8*). The IgM fraction from the serums of nonrheumatoid patients was prepared similarly. Neutralizing antibody to HSV was not detected in any of these preparations. All serums, except sources of complement, were heated at 56°C for 30 minutes.

Infectious VA complexes, prepared as described (*3*), were incubated with RF or IgM from nonrheumatoid patients. Portions from each reaction mixture were then removed and incubated with specific antibody to immunoglobulins or with complement. The reaction mixtures were assayed for surviving virus and the percentage of neutralization as compared to appropriate controls was calculated (*3*).

Incubation of rabbit anti-HSV IgG (untreated) with HSV resulted (Table 1) in the formation of infectious HSV-IgG complexes that were highly susceptible to neutralization by antiserum to rabbit IgG (anti–rabbit IgG), moderately susceptible to guinea pig complement, but not susceptible to antiserum to human IgM (anti–human IgM). Incubation of infectious HSV-IgG with human RF did not result in neutralization, but made the complexes susceptible to neutralization by anti–human IgM and enhanced the amount of neutralization produced by complement. Virus sensitized with IgG treated with 2-mercaptoethanol was completely resistant to neutralization by complement and anti–human IgM, but exposure to RF made the virus which had been sensitized with 2-mercaptoethanol–treated IgG highly suspectible to neutralization by both complement and anti–human IgM. The degree of susceptibility to complement (*9*) and anti-IgM was related to the concentration of RF. RF had no effect on the neutralization of unsensitized virus.

Incubation of HSV with the papain-derived Fab I fragment of rabbit anti-HSV IgG (Table 1) also resulted in the formation of infectious VA complexes (*3*). Incubation with RF, however, did not increase the susceptibility of these complexes to neutralization by anti–human IgM or complement. This suggests that in the absence of the Fc portion of anti-HSV IgG, RF failed to attach to the infectious complexes.

To see whether RF could lead to the enhancement of virus neutralization in a homologous system, human anti-HSV IgG and RF were isolated from the serum of the same individual. Serum

Table 1. Effect of rheumatoid factor (RF) on the neutralization of virus sensitized with rabbit IgG. Infectious VA complexes were prepared by incubating $10^{5.5}$ to $10^{6.0}$ plaque-forming units (PFU) of HSV with equal volumes of appropriate dilutions of anti-HSV IgG or normal serum for 2 hours at 37°C. Portions were removed, diluted 1 to 5, and incubated at 37°C for 15 minutes with equal volumes of RF or IgM from nonrheumatoid individuals. Samples were then removed and incubated for another 15 minutes at 37°C with equal volumes of antiserum to IgG (diluted 1 : 12.5), antiserum to IgM (diluted 1 : 12.5), or complement (30 CH_{50}/ml). Samples incubated with normal serum served as controls. The virus titer in each of the reaction mixtures was determined and the percentage of neutralization as compared to appropriate controls was calculated. The data represents the average of two to four experiments. (CH_{50} is a hemolytic unit of complement.)

Reaction mixture		Titer of virus in surviving fraction (PFU/ml, log 10)	Percentage of virus in surviving fraction neutralized by		
Rabbit anti-HSV IgG*	Human IgM		Anti–rabbit IgG	Anti–human IgM	Guinea pig complement
Untreated	Non-RF (1:8)†	4.9	99	0–20	84
Untreated	RF (1:8)	4.9	99	94	96
2-ME‡	Non-RF (1:8)†	5.0	99	0	0
2-ME‡	RF (1:4)	4.7	99	94	98
2-ME‡	RF (1:8)	5.0	99	94	94
2-ME‡	RF (1:80)	5.0	99	37	37
2-ME‡	RF (1:160)	5.0		0	0
Fab	Non-RF (1:8)†	5.3	94	0–20	0
Fab	RF (1:8)	5.3	94	0–20	0
Control§	Non-RF (1:8)†	6.0	0	0–20	0
Control§	RF (1:8)	6.0	0	0–20	0

* Each reaction mixture contained 140 μg of IgG per milliliter. † IgM (diluted 1 : 8) from nonrheumatoid patient. ‡ IgG was treated with 2-mercaptoethanol and iodoacetamide. § Serum (diluted 1 : 25) from normal rabbits.

Table 2. Effect of rheumatoid factor (RF) on the neutralization of virus sensitized with human IgG.

Reaction mixture		Titer of virus in surviving fraction (PFU/ml, log 10)	Percentage of virus in surviving fraction neutralized by		
Human anti-HSV IgG (dilution)	Human IgM*		Anti-human IgG	Anti-human IgM	Human complement
1:25	Non-RF†	4.4	95	20	37
1:25	RF	4.3		94	95
1:50	Non-RF†	5.0	97	20	20
1:50	RF	5.0		96	92
1:100	Non-RF†	5.7	60	20	20
1:100	RF	5.4		60	50
1:200	Non-RF†	5.8	37	20	0
1:200	RF	5.8		20	0
Control‡	Non-RF†	5.8	0	20	0
Control‡	RF	5.8	0	20	0

* Diluted 1 : 5 in all reaction mixtures. † IgM from nonrheumatoid patient. ‡ IgG (diluted 1 : 25) from patient lacking antibody to HSV.

from an individual lacking both RF and antibody to HSV served as the source of complement. The data in Table 2 show that infectious complexes composed of HSV and human anti-HSV IgG were not susceptible to neutralization by anti–human IgM and only slightly susceptible to neutralization by human complement. After incubation with RF, however, the infectious complexes were highly susceptible to neutralization by both anti–human IgM and human complement. The amount of neutralization produced by anti–human IgM and complement was related to the concentration of anti-HSV IgG used to sensitize the virus.

Our experiments showed that incubation of RF with infectious HSV-IgG complexes failed to produce neutralization. However, the demonstration that specific anti–human IgM was able to neutralize infectious complexes that had been incubated with RF showed that RF had attached to these complexes. Moreover, the newly formed infectious HSV-IgG-RF complexes were quite susceptible to neutralization by complement. Our findings suggest that the interaction of RF with IgG provided new sites for the attachment of complement (*10*) and that increased coverage of the surface of the virion by the components of complement was probably responsible for neutralization (*4*). The ability of RF to enhance virus neutralization in the presence of complement suggests that RF might play a role in the host's reaction to virus infections.

Although in our experiments RF enhanced complement-mediated neutralization, it is possible that RF might be inhibitory to the complement-mediated neutralization of viruses sensitized with different classes or quantities of immunoglobulins. Schmid *et al.* (*10*) showed that in the red blood cell system whether RF enhanced or inhibited complement fixation depended on the concentration of sensitizing IgG. Recently several investigators reported that RF interfered with the fixation of complement by certain viruses (*11*). Whether complement competitively interferes with the fixation of RF remains to be determined. Apart from the role of RF in virus neutralization, the demonstration that RF or complement or both can attach to VA complexes suggests that not only VA complexes (*2*) but VA-complement complexes, VA-RF complexes, and VA-RF-complement complexes should be considered in the etiology and pathogenesis of immune-complex disease.

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Transneuronal Transfer of Radioactivity in the Central Nervous System

Abstract. *After injection of tritiated amino acid into the mouse eye, radioactivity appeared in the contralateral visual cortex, indicating that some material had been transferred from optic axons to lateral geniculate neurons. The radioactivity in the cortex was about 2 percent of that arriving in the geniculate, and most of it was contained in material that appeared to be protein.*

The idea that materials may pass from one nerve cell to another is closely related to the concept that the neuron has a "trophic" action on other cells, an action which enables it to "initiate or control molecular modification in the other cell[s]" (*1*). In demonstration of this trophic effect, it has been shown that, when a nerve fiber degenerates, its postsynaptic elements (or, in the case of a sensory nerve fiber, its sensory receptors) become severely deranged in both structure and function, and sometimes even disappear (*2*). For example, removal of the eye, which causes degeneration of the primary optic fibers, is followed by atrophy of the cells of the lateral geniculate body on which the optic fibers end (*3*). There is some indirect evidence that the trophic effects are mediated by the release from the nerve cell of substances other than the usual synaptic transmitters (*1*).

Direct evidence for the transneuronal