intracellular viscosity is not significantly greater than an aqueous solution of the hemoglobin at a similar concentration. Using the Stokes-Einstein equation and a value of 55 Å for the molecular diameter (*17*) gives viscosities of 1.94 centipoise and 1.56 centipoise before and after hemolysis. These results support the view that the protoplasmic viscosity is not very different from that of pure water; most of the differences that do exist can be explained by the high concentration of protein present. The results differ, however, from the general conclusions of Keith and Snipes (*7*) who found large values for the intracellular viscosities of a variety of cells. The difference may reflect differences in the cells tested or in the interpretation of the data, as has been suggested (*6*). In general, the use of ^{13}C NMR to study both intracellular and extracellular proteins should prove valuable for determining differences between the in vivo and in vitro environments of enzymes.

R. E. London, C. T. Gregg
N. A. Matwiyoff

Los Alamos Scientific Laboratory,
University of California,
Los Alamos, New Mexico 87544

References and Notes

1. N. A. Matwiyoff and T. E. Needham, *Biochem. Biophys. Res. Commun.* **49**, 1158 (1972).
2. R. T. Eakin, L. O. Morgan, C. T. Gregg, N. A. Matwiyoff, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **28**, 259 (1972).
3. J. D. Robinson, N. M. J. Birdsall, A. G. Lee, J. D. Metcalfe, *Biochemistry* **11**, 2903 (1972).
4. N. A. Matwiyoff and B. F. Burnham, *Ann. N.Y. Acad. Sci.* **206**, 365 (1973); T. E. Needham, N. A. Matwiyoff, T. Walker, H. P. C. Hogenkamp, *J. Am. Chem. Soc.* **95**, 5019 (1973); D. T. Browne, G. L. Kenyon, E. L. Packer, H. Sternlicht, D. M. Wilson, *Biochem. Biophys. Res. Commun.* **55**, 42 (1972); *J. Am. Chem. Soc.* **95**, 1316 (1974); M. W. Hunkapiller, S. H. Smallcombe, D. R. Whitaker, J. H. Richards, *Biochemistry* **12**, 4733 (1973); *J. Biol. Chem.* **248**, 8306 (1973); I. M. Chaiken, M. H. Freedman, J. R. Lyerla, J. S. Cohen, *ibid.*, p. 884.
5. N. A. Matwiyoff and D. G. Ott, *Science* **181**, 1125 (1973).
6. E. D. Finch and J. F. Harmon, *ibid.* **186**, 157 (1974).
7. A. D. Keith and W. Snipes, *ibid.* **183**, 666 (1974).
8. C. Tesar and D. Rittenberg, *J. Biol. Chem.* **170**, 35 (1947).
9. J. N. Ashley and C. R. Harrington, *J. Chem. Soc.* (1930), p. 2586.
10. R. L. Vold, J. S. Waugh, M. P. Klein, D. E. Phelps, *J. Chem. Phys.* **48**, 3831 (1968).
11. M. O. Dayhoff, Ed., *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Washington, D.C., 1969).
12. J. B. Stothers, *Carbon-13 NMR Spectroscopy* (Academic Press, New York, 1972), p. 479.
13. P. Vergamini, private communication.
14. A. Allerhand, D. Doddrell, R. Komorowski, *J. Chem. Phys.* **55**, 189 (1971).
15. D. Doddrell, V. Glushko, A. Allerhand, *ibid.* **56**, 3683 (1972).
16. R. Visscher and F. R. N. Gurd, *Fed. Proc.* **33**, 1441 (1974).
17. M. F. Perutz, *Proc. R. Soc. London Ser. B Biol. Sci.* **173**, 113 (1969).
18. This work was performed under the auspices of the Atomic Energy Commission.

25 November 1974; revised 8 January 1975

Inhibition of Immune Responses in vitro by Specific Antiserums to Ia Antigens

Abstract. *Mouse antiserums prepared against Ia antigens, which are products of I (immune reponse) region genes of the H-2 complex, can inhibit both primary (immunoglobulin M) and secondary (immunoglobulin G) immune responses in vitro by mouse spleen cultures to heterologous erythrocytes. Antiserums directed specifically at products of either the H-2K or H-2D loci have no effect on this response.*

Recently we and others have described a system of antigens, Ia, which is controlled by the I (immune response) region of the H-2 gene complex (*1–4*). These antigens are expressed only on a subset of lymphocytes, which led to early conclusions that Ia antigens were confined to either thymus-derived (T) cells (*3, 4*), or bone marrow–derived (B) cells (*2*). We have shown that Ia antigens are expressed both on T and B cells (*5*).

If Ia antigens are related either to *Ir* gene products or to the cooperation factors postulated by Katz (*6*), then one might predict inhibition of immune response in vitro by antiserum to Ia antigens, by direct blockage of either the antigen receptor or of the cooperation sites on the cell membrane by antibodies in the serum. Pierce *et al.* (*7*) have shown that polyspecific H-2 alloantiserums, which could contain antibodies to Ia, inhibit primary responses in vitro to sheep red blood cells (SRBC).

Using carefully defined antiserum to Ia, antiserum to H-2K, or antiserum to H-2D, we have been able to show inhibition of the secondary response to burro red blood cells (BRBC) in vitro, as well as inhibition of the primary response to SRBC.

All mice used were raised in the mouse colony of the Department of Human Genetics, University of Michigan. Antiserums specific for Ia antigens were produced by reciprocal immunization of A.TH and A.TL mice (*1*). These strains are identical in their H-2K and H-2D regions, and differ only in the I and S (serum protein) regions of the H-2 complex. All serums used came from a single pool that was well characterized for cytotoxic activity. These serums contain multiple antibodies to antigenic determinants mapping in the I region. Antiserum to $H\text{-}2K^s$ was prepared in $(A \times A.AL)F_1$ mice by immunization with tissue from A.TL mice. Two antiserums reacting with H-2D specificities were used as controls: (i) $(C3H \times B10)F_1$ antiserum to C3H.Q, an antiserum to H-2.13, which might contain antibodies to I^q region antigens but could not contain antibody to I^k region antibodies because the $H\text{-}2^k$ haplotype is present in the recipient; or (ii) $(B10 \times AKR.M)F_1$ antiserum to B10.A, which could also contain antibody to I^d region antigens but also is blocked for I^k region antigens by the presence of AKR.M in the recipient. Antiserums were diluted 1 : 2 and sterilized by filtration before they were used in cultures.

Mouse spleen cell cultures were established by a modification of the method of Mishell and Dutton (*8*). Primary cultures were made with SRBC from a single animal and assayed on day 4 for direct plaque-forming cells (PFC) by a modification of the method of Jerne and Nordin (*9*). For secondary responses, spleen donors were immunized at least 3 weeks before use with 0.1 ml of a 10 percent suspension of BRBC. Cultures were stimulated with BRBC and assayed on day 4 for both direct and indirect PFC. For cultures treated with antiserum, two procedures were used. In the primary responses, 100 μl of the sterile diluted antiserum was added to the cultures on day 0. In the secondary responses, spleen cells were treated before culture with a single concentration of antiserum (usually 1 : 2) for 20 minutes at room temperatures, and then placed on ice for 20 minutes. The cells were washed four times and then cultured with BRBC.

After adding either of the two specific antiserums to Ia to unprimed cell cultures we observed a depression of the number of direct PFC recovered. However, in many experiments there were suppressive effects of normal serum, which did not seem to be related to a particular serum, since the same pool of normal serum might be suppressive one day and inactive another. We have pooled the data from the experiments on primary responses from all treatments at concentrations of serum greater than 1 : 1000. Responses of cultures treated with nor-

mal serum, antiserum to H-2K, and antiserum to H-2D were 158, 211, and 174 PFC per 10^6 recovered, respectively. These results are not significantly different as judged by an F test ($P > .1$). Treatment with antibody to Ia reduced the response to 52 PFC per 10^6 cells, a significant reduction ($P < .001$). The percentage of viable cells recovered was the same in all treatment groups.

Effects on the secondary response to BRBC were measured by first treating the cells with antiserum in the absence of complement. We used BRBC because the background response in unstimulated cultures is very much less than with SRBC. An early experiment in which the antiserum was added when the culture was started is shown in Fig. 1. There was greater inhibition by the antiserum to Ia compared to a control antiserum to H-2K at all concentrations tested when IgG PFC are examined. Less difference was seen in the IgM response. We observed variation in the effects of normal serum on such cultures. This problem could be avoided by treating the spleen cells with antiserum prior to starting the cultures. Two experiments with cells subjected to such prior treatment with antiserums are shown in Fig. 2. For both antiserums to Ia, the IgG response was suppressed, while antiserum to H-2K had no effect. The IgM response was also strongly inhibited. This is in contrast to the experiments in which antiserum was added to the cultures undergoing a secondary response. In no case were the antiserums to H-2K or to H-2D controls inhibitory.

We undertook these experiments to test the possibility that Ia antigens are associated with Ir gene products. The experiments with xenogenic blood cells were initiated as controls, on the basis that no antibodies would be produced against the I region receptors for these antigens since both strains are responders. Extrapolating from the guinea pig data on blocking of antigen-induced proliferation (*10*), we had expected that we would be able to interfere with only those responses in which one of the pair of immunizing strains was a responder and the other a nonresponder. However, Tyan (*11*) has reported that immune mouse serums can inhibit both antigen- and mitogen-induced proliferation in the mouse. His data are difficult to relate to ours because his antiserums were prepared in such a way that they could have con-

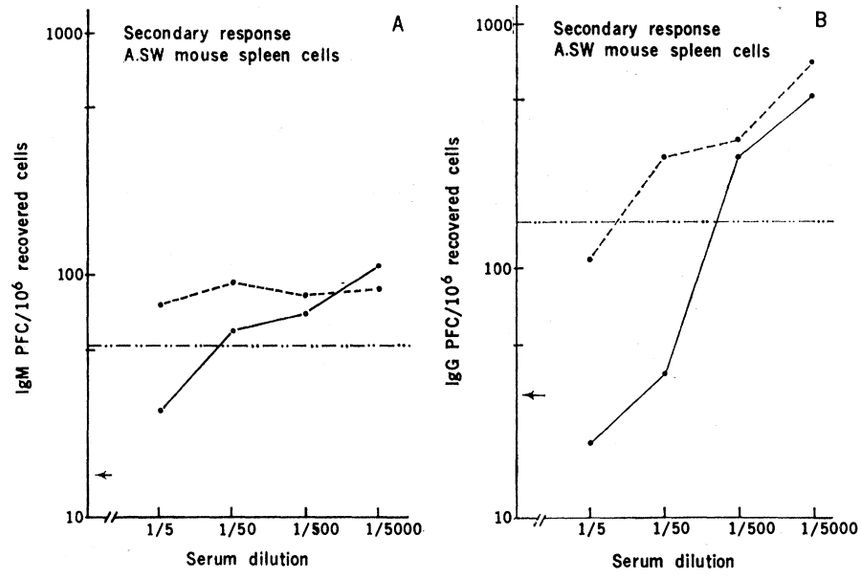

Fig. 1. Effect of the addition of antiserum to Ia on cultures of BRBC-primed cells. The antiserum dilution (100 μl) was added to each 1 ml of culture concentration. All experiments were performed in triplicate. The dot-and-dash (horizontal) lines represent the response in the absence of serum; the arrow represents the response in the absence of antigen. ●—●, Antiserum to Ia^s; ●----●, antiserum to $H\text{-}2K^s$. (A) IgM response; (B) IgG response.

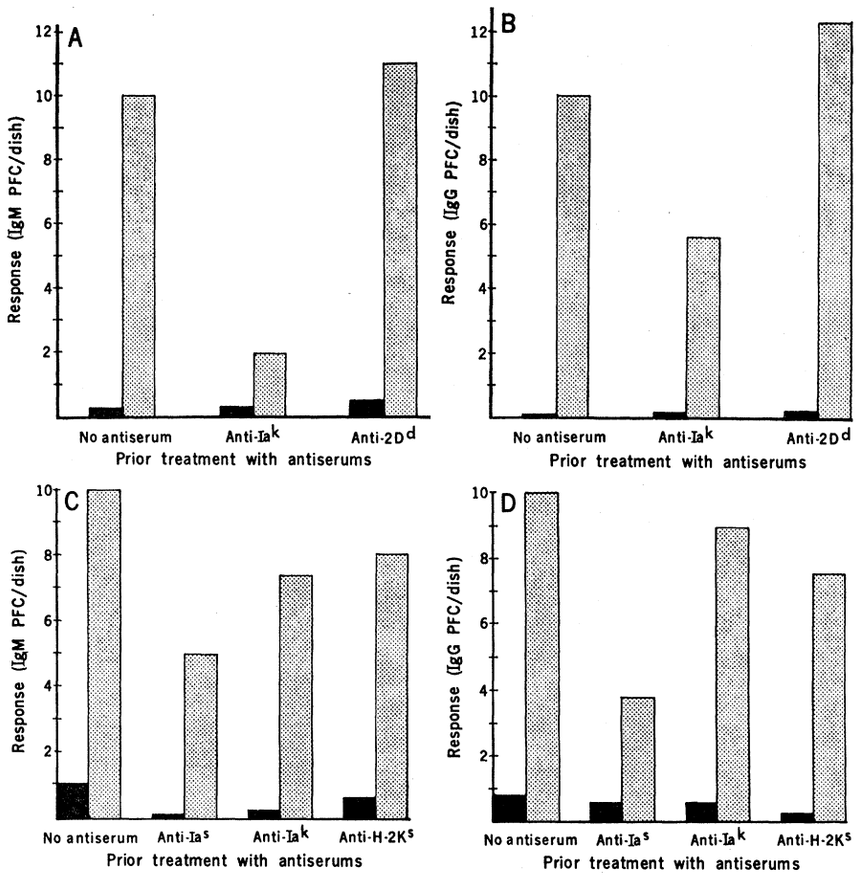

Fig. 2. Effect of prior treatment with antiserum on cultures of BRBC-primed cells. Cells were first treated with the indicated antiserum without complement and cultured without (dark bars) and with (stippled bars) BRBC. Results are plotted on a scale in which controls are normalized to 10 PFC per dish. (A) IgM response of A.TL cells; control, 1520 PFC per culture. (B) IgG response of A.TL cells; control, 4780 PFC per culture. (C) IgM response of A.SW cells; control, 1568 PFC per culture. (D) IgG response of A.SW cells; control, 9400 PFC per culture.

tained antibodies to H-2, Ia, and other cell-surface antigens (non-H-2).

Pierce *et al.* (*7*) have investigated the ability of antibodies to both H-2 and non-H-2 to inhibit in vitro primary responses to GAT_{10} (a linear polymer of glutamic acid, alanine, and tyrosine) and SRBC. They found that antiserums directed against only H-2D have no effect on the response, in agreement with our results. They also observed that antiserums which can contain antibody to Ia in addition to antibody to H-2K inhibit both IgM and IgG primary responses. An antiserum, which they assume to be a specific antibody to H-2K without any possible Ia antibodies, could in fact contain antibodies to $I\text{-}C^k$. The antiserum to H-2K that we used in our study cannot contain these antibodies (*12*). Using this antiserum we found no inhibition of the response. Pierce *et al.* (*7*) observed no effect of a specific antiserum to Ia on primary IgM responses. That serum, which lacked cytotoxicity, was obtained from early bleedings of animals that produced the antiserums that we used in our experiment. Later bleedings of the same group of animals yielded highly cytotoxic serums, which produced a marked inhibition of the primary IgM response. When we added the antiserum directly to the culture as in the experiments of Pierce *et al.*, instead of treating the cells with antiserum before starting the culture, we observed little inhibition of the secondary IgM, but the IgG response was inhibited.

We emphasize that simply attaching any antibody to the cell surface does not inhibit an immune response, as shown by antiserums directed at H-2D or H-2K specificities. Antiserums directed at I region products do alter the immune response, as would be expected if Ia antigens were either cooperative factors or the antigen receptors themselves. An important remaining question with regard to the mode of inhibition by antiserums to Ia is whether prior treatment permanently alters the capacity of the cells to respond to antigen. It would be expected that cells with antibody bound to them would lose the antibody-antigen complexes either by simply shedding the antigens, or by capping and pinocytosis. If the cells can rapidly generate new antigens, then one would predict that preliminary treatment with antiserums would alter only the kinetics of the response, by delaying initiation —that is, recognition or cooperation. In some initial experiments we have observed such an alteration of the kinetics; in 4-day-old cultures there was a marked specific suppression by antiserum to Ia, and in 5-day-old cultures there was no significant inhibition by antiserum to Ia.

JEFFREY A. FRELINGER
Department of Human Genetics, University of Michigan, Ann Arbor 48104

JOHN E. NIEDERHUBER
Department of Surgery and Microbiology, University of Michigan

DONALD C. SHREFFLER
Department of Human Genetics, University of Michigan

References and Notes

1. C. S. David, D. C. Shreffler, J. A. Frelinger, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2509 (1973).
2. D. H. Sachs and J. L. Cone, *J. Exp. Med.* **138**, 1289 (197→ G. Hämmerling, B. D. Deak, G. Mauve, U. Hämmerling, H. O. McDevitt, *Immunogenetics* **1**, 68 (1974).
3. V. Hauptfeld, D. Klein, J. Klein, *Science* **181**, 167 (1973).
→ D. Gotze, R. A. Reisfield, J. Klein, *J. Exp. Med.* **138**, 1003 (1973).
→ J. A. Frelinger, J. E. Niederhuber, C. S. David, D. C. Shreffler, *ibid.* **140**, 173 (1974).
→ D. H. Katz, T. Hamoka, M. E. Dorf, P. H. Mauer, B. Benaceraf, *ibid.* **138**, 734 (1973).
→ C. W. Pierce, J. A. Kapp, S. M. Solliday, M. E. Dorf, B. Benaceraf, *ibid.* **140**, 921 (1974).
→ R. I. Mishell and R. W. Dutton, *ibid.* **126**, 423 (1967); the medium was modified by the addition of 0.01*M* Hepes buffer and 2×10^{-5}*M* 2-mercaptoethan → [R. E. Click, L. Benck, B. J. Alter, *Cell. Immunol.* **3**, 264 (1972)].
→ N. K. Jerne and A. A. Nordin, *Science* **140**, 405 (19→ D. W. Dresser and H. W. Wortis, *Nature (Lond.)* **208**, 859 (196→ W. W. Bullock and E. Moller, *Eur. J. Immunol.* **2**, 514 (1972).
→ E. M. Shevach, W. E. Paul, I. Green, *J. Exp. Med.* **139**, 661 (197→ E. M. Shevach, I. Green, W. E. Paul, *ibid.*, p. 679.
→ M. L. Tyan, *Cell. Immunol.* **10**, 450 (1974).
12. Pierce's serum was produced by immunization of A strain mice with tissue from A.TL mice. Our antiserum was produced in $(A \times A.AL)F_1$ mice by immunization with A.TL tissue.
13. Supported by PHS program project 2-P01-GM15419-07, NIH grant R01-AI-11962-01, the Kidney Foundation of Michigan, a Damon Runyon grant DRG-1260, a Jane Coffin Childs fellowship to J.A.F., and career development awards to J.E.N. and D.C.S. We thank A. Burt for technical asistance, and Dr. Peter Smouse for statistical advice.

25 September 1974; revised 18 November 1974 ■

Synaptic Organization of the Amine-Containing Interplexiform Cells of the Goldfish and Cebus Monkey Retinas

Abstract. *Fluorescence microscopy has revealed a new type of amine-containing retinal neuron, the interplexiform cell, that extends processes in both plexiform layers. After intravitreal injection of 5,6-dihydroxytryptamine in goldfish and* Cebus *monkey, the processes of these cells can be identified by electron microscopy. In goldfish, the processes are pre- and postsynaptic to amacrine cells in the inner plexiform layer and presynaptic to bipolar and horizontal cells in the outer plexiform layer. Interplexiform cells thus provide an intraretinal centrifugal pathway from inner to outer plexiform layers.*

It is generally accepted that certain catecholamines act as neurotransmitters in the vertebrate central nervous system (*1*). In the retina, the fluorescence method of Falck and Hillarp (*2*) has shown that there are a small number of dopamine-containing neurons whose perikarya are found most often among the amacrine cells (*3*). At least some of these neurons appear to be of a type not previously recognized since in some animals (teleost fish and New World monkeys) they are observed to extend processes widely in both the inner and outer plexiform layers (*4*). Cells with similar morphological characteristics have recently been observed in Golgi preparations of cat, Old World monkey, and dolphin eyes and have been called interplexiform cells (*5*). The synaptic connections made by these neurons are not known.

Catecholamine-containing neurons have efficient mechanisms for the uptake and concentration of both the natural transmitters and certain analogs (*6*). Some of these analogs alter the appearance of the fine structure of the neurons taking up the drug, enabling the processes and perikarya of the amine-containing cells to be readily recognized by means of electron microscopy (*7*). We have examined the effects of a number of these drugs on the retina and report here experiments in which one such drug, 5,6-dihydroxytryptamine (5,6-DHT), was injected into the vitreous humor of goldfish (*Carassius auratus*) and *Cebus* monkey (*Cebus capucinus*) eyes. The synapses made by and onto the amine-containing cells and their processes were studied, and the synaptic organization of the amine-containing interplexiform cells was deduced. This method, which permits identification