Antiserum to Immunoglobulin A: Inhibition of Cell-Mediated Demyelination in Tissue Culture

Abstract. *Lymph node cells from rats immunized with sciatic nerve in Freund's adjuvant demyelinate trigeminal ganglion cultures. Rabbit antiserum to rat immunoglobulin A blocks this cytodestructive event. The antiserum may act by combining with IgA on the lymphoid cell surface, preventing the interaction of cell-bound antibody and tissue antigen.*

Several experiments are now recorded in which lymph node cells from animals immunized with a given tissue antigen have destroyed a culture of that tissue (*1*). The lesion produced in vitro by transferred lymphoid cells serves as a model for that produced in animals by passively transferred, sensitized lymphoid cells. In the lesions of delayed hypersensitivity in vivo, the infiltrating lymphocytes proliferate while undergoing a morphologic alteration to monocytoid cells (*2*). Sensitized lymphocytes exposed to their specific protein antigen in vitro undergo similar alterations 48 to 72 hours after exposure (*3*).

The nature of delayed hypersensitivity is not understood, but lymphocytes are involved and may acquire specificity during immunization. Thymectomized animals are deficient in lymphocytes, show an impaired capacity to express delayed hypersensitive responses, and have a subnormal amount of IgA (immunoglobulin A) in their serum (*4*). A similar defect in serum IgA has been noted in humans with ataxia-telangiectasia, a disease in which thymic development is abnormal, lymphocytes are low, and delayed hypersensitive responses are defective (*5*). In ataxia-telangiectasia, the considerable numbers of IgA-producing cells in the marrow (*6*) suggests that the low serum IgA in this disease reflects a disorder in antibody mobilization rather than in synthesis. It is not known if thymectomized animals have normal numbers of IgA-producing cells.

We have reported that lymph node cells from rats immunized with rat sciatic nerve in Freund's adjuvant demyelinate cultures of rat trigeminal ganglion (*7*). Node cells acquire this capacity 1 week after immunization. Cells taken 7 to 10 days after immunization show the greatest capacity to destroy myelin; cytodestructive capacity wanes slowly thereafter. Demyelination begins after the cells and cultures have been in contact for 48 to 72 hours. The cytodestructive effect is selective. Only myelin is destroyed; the axons which it envelops are left intact. The effect is specific. Cells from rats immunized with other antigens, even white matter from the central nervous system, will not destroy the cultures. A single exception has been found. Cells from rats immunized with kidney will often demyelinate the cultures, a probable indication of shared tissue antigen. Serum taken 1 to 2 weeks after immunization with sciatic nerve rarely destroys myelin. Serum taken more than 2 weeks after immunization will demyelinate about one third of the cultures.

We have prepared rabbit antiserums to various rat immunoglobulins (*4*). In the experiments described here a monospecific antiserum directed against rat IgA was added to the cultures along with the sensitized cells.

Trigeminal ganglia were excised from fetal rats (17 to 18 days' gestation) and grown on collagen-coated coverslips in Maximow double-coverslip assemblies. The cultures were fed one drop of a medium which contained equal proportions of human placental serum, chicken embryo (9 days old) extract, and Tyrode's solution. The final medium also contained glucose, 1.1 to 1.2 percent, and penicillin, 5000 units per milliliter. Medium was changed twice weekly (*8*). Myelin appeared during the 2nd week and increased in amount until the 5th week. Cultures with abundant myelin (Fig. 1*a*) were selected for use.

Sprague Dawley rats (150 to 200 g, female) received 0.25 ml of a 20-percent suspension of rat sciatic nerve in Freund's adjuvant in one hind foot pad. Seven to twelve days later, the draining inguinal node was excised, trimmed, and teased apart in Tyrode's solution. The liberated lymphocytes were centrifuged at 700 rev/min for 10 minutes, and the supernatant was removed with a Pasteur pipet.

The cells for control cultures were then suspended in the standard feeding medium. The cells for experimental cultures were suspended in feeding medium which contained rabbit antiserum to rat IgA in place of placental serum. Each culture received one drop of the final medium which contained 3 to 5 $\times$ 10^6 cells. Occasional cultures became contaminated after addition of lymph node cells and were discarded. A limited number of experiments were done with rabbit antiserum to rat IgG or rabbit antiserum to rat α_2-macroglobulin being substituted for antiserum to IgA.

Demyelination occurred in 26 percent of the cultures given antiserum to IgA, and in 69 percent of the controls

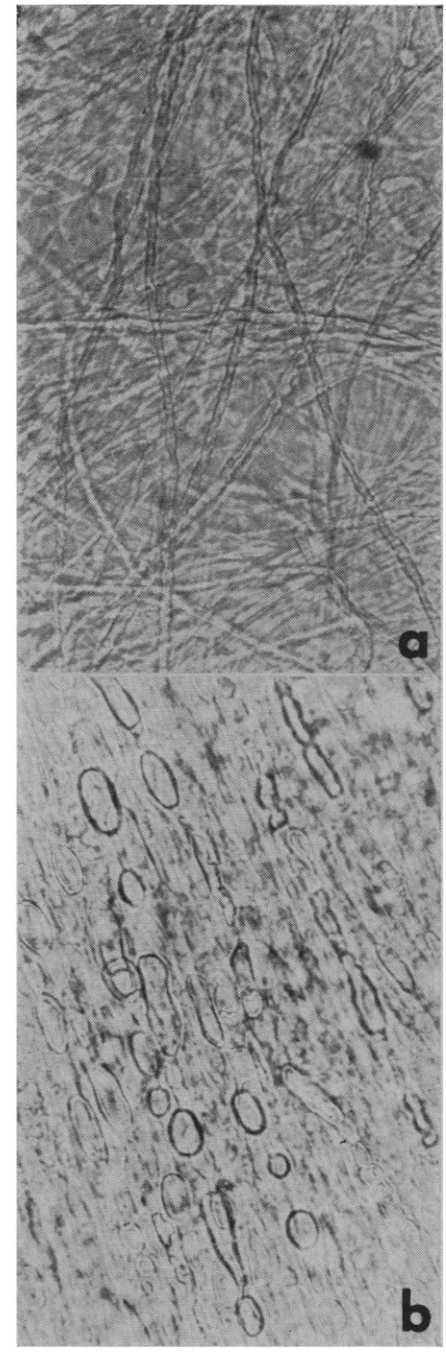

Fig. 1. (*a*) Representative myelinated trigeminal ganglion culture. There is an abundance of myelin ($\times$ 270). (*b*) Culture 96 hours after addition of sensitized lymphocytes. The myelin is breaking down ($\times$ 400).

Table 1. Inhibition of demyelination by antiserum to IgA. The proportion of cultures undergoing demyelination when exposed to sensitized lymph node cells is compared to the proportion undergoing demyelination when exposed to cells plus rabbit antiserum to IgA.

Day node cells taken after immunization	Cultures demyelinated	
	Node cells plus rabbit antiserum to rat IgA	Node cells only
7	*0/2	3/3
8	1/2	2/2
9	0/3	3/3
10	2/2	1/1
11	0/2	3/6
12	0/2	2/2
12	1/3	3/6
12	1/3	1/3

* One culture showed incipient degeneration in two fibers.

(Table 1). This difference is significant at 0.05 by the chi-square test. The incidence of demyelination in the controls corresponds closely with our accumulated experience to date: 37 out of 57 (65 percent) cultures that had been given lymphocytes taken from animals 7 to 12 days after immunization have demyelinated. Demyelination was first evident at 72 hours and was extensive by 96 hours when the experiment was terminated (Fig. 1*b*).

Data with other specific antiserums suggest that this effect is specific. Addition of specific rabbit antiserum to rat IgG to the feeding medium has failed to block the myelin-destructive capacity of sensitized cells. Addition of a rabbit antiserum to rat α_2-macroglobulin serum has similarly been ineffectual.

Lymph node cells that have been treated with high-frequency sound do not demyelinate the cultures (*9*); this suggests that some function of the intact cells is necessary for demyelination to occur. They could elaborate an IgA class antibody to a peripheral nerve constituent. This antibody might attack the myelin directly or become firmly attached to lymphocytes, making them active cells. Alternatively, the sensitized cells might have had a cytodestructive antibody of the IgA class attached to their surfaces in the lymph nodes. In any of the above-mentioned cases, an antiserum to IgA would act by interposing itself between the IgA antibody to nerve and the peripheral nerve antigen.

A third possibility is suggested by the experiments of Sell and Gell (*10*). They found that antiserum to allotype (directed against genetically determined antigenic sites on rabbit immunoglobulins) added to rabbit lymph node cell cultures would trigger morphologic alteration and proliferation of the type seen after addition of specific antigen to sensitized cells. Possibly our rabbit antiserum to IgA triggered a similar response in the cells added to our cultures, directing them away from the cytodestructive response they make ordinarily. Finally, it is possible that antiserum to IgA binds to receptor sites on the peripheral nerve cultures impeding access of the sensitized cell or its active product to the peripheral nerve antigen.

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Polysomes and Protein Synthesis in Cells Infected with a DNA Virus

Abstract. *In HEp-2 cells infected with herpes simplex virus the rate of protein synthesis at first decline, is stimulated between 4 and 8 hours after infection, and progressively and irreversibly declines from 9 to 16 hours later. The increase and decrease in rates coincide with corresponding changes in the amounts of cytoplasmic polysomes and amounts of labeled amino acids in nascent peptides bound to polysomes. The data indicate that (i) early and late inhibition and intervening stimulation of protein synthesis are due to the corresponding breakdown and formation of polysomes, and (ii) the bulk of viral proteins is probably made on cytoplasmic polysomes.*

Herpes simplex virus inhibits the uptake of precursors into DNA, RNA, and proteins (*1*) immediately after infection. With regard to protein synthesis in infected HEp-2 cells we now report two findings. First, the pattern of protein synthesis in the cytoplasm of infected cells consists of (i) an initial decline lasting approximately 3 hours, (ii) a period of stimulated synthesis from about 4 through 8 hours after infection, and (iii) a progressive, irreversible decline. This pattern corresponds to the pattern of amino acid uptake into peptides of whole infected cells reported previously (*1*). Second, the stimulation and inhibition of protein synthesis coincide with increases and decreases, respectively, in the amounts of cytoplasmic polysomes and with corresponding changes in the uptake of labeled amino acids into nascent peptides. The data thus indicate that breakdown and formation of cytoplasmic polysomes account for the pattern of protein synthesis in infected cells.

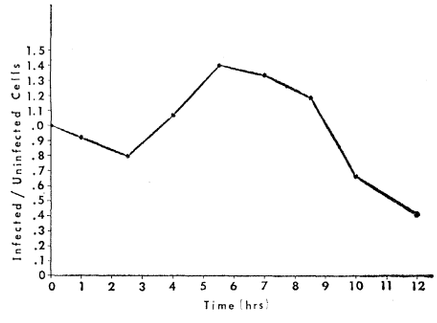

Fig. 1. Variation in specific activity of trichloroacetic acid–insoluble peptides extracted from the cytoplasm of cells pulse-labeled with C^{14}-amino acids at different times after infection with herpes simplex virus. The specific activity is corrected for that obtained from uninfected cells.