## Heterologous Antilymphocyte Globulin: Studies in vitro

Abstract. *Incubation of dog-lymphocyte cultures with globulin from antiserum to lymphocytes resulted in a greater percentage of transformed cells than incubation with phytohemagglutinin. Viability counts demonstrated that this globulin and phytohemagglutinin had approximately equal degrees of cytotoxicity.*

Heterologous antiserum to lymphocytes (ALS) and the globulin fraction of antiserum to lymphocytes (ALG) have been used successfully in vivo to prolong homograft survival (*1, 2*). Starzl *et al.* (*3*) have reported that homograft survival may occur without a significant decrease in peripheral lymphocytes. Hence, the mechanism of increased homograft survival seen with these preparations remains speculative. There is evidence to suggest that the mechanism is due to some nonspecific action. For example, spleens from patients treated with ALG show an increase in number of large pyroninophilic cells (*3*). Furthermore, Calne (*4*) reported that phytohemagglutinin (PHA) plus azathioprine is more effective in prolonging homograft survival than azathioprine alone.

In an attempt to elucidate the mechanism of action of ALS and ALG, we used the following methods in vitro. Cultures of peripheral lymphocytes taken from eight dogs during the primary immune response and from four dogs during the secondary immune response were studied. For the primary immune response 0.5 ml of a 10 percent suspension of washed sheep erythrocytes was injected both intravenously and intraperitoneally. Two to three weeks later, the dogs received a second intravenous and intraperitoneal injection of sheep erythrocytes to stimulate a secondary immune response. The lymphocytes were isolated, and cultures were prepared according to a modification of the technique described by Bach and Hirschhorn (*5*). Horse ALG (supplied by Drs. Marchioro and Starzl) and normal horse globulin were added to the cultures. Hence each culture tube contained 4 ml of medium 199 (Hank's base) to which penicillin, streptomycin, and 10 percent fetal calf serum were added. The ALG was obtained by precipitation with ammonium sulfate; the normal horse globulin (Pentex Laboratories) was obtained by alcohol fractionation. Both of the globulins were added to the culture tubes in 0.1-ml volume as a 4.5 percent solution. Control cultures received medium only or medium plus phytohemagglutinin. A portion of the cells was removed from each culture at 24 hours, and viability was determined by a dye exclusion technique with trypan blue. At 72 hours, the cells were harvested and the number of blast forms were counted. Since viability studies were not made at this time, all intact cells were counted.

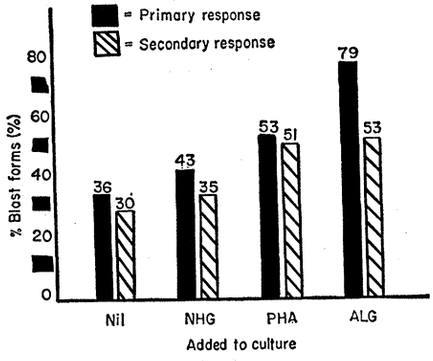


Fig. 1. Percentage of blast forms in lymphocyte cultures. Mean values from the primary response in eight dogs and from the secondary response in four dogs. *NHG*, normal horse globulin.

Control cultures from these immunized dogs showed a variable but significantly increased number of blast forms. Cultures of lymphocytes from "normal" dogs showed a greater number of blast forms (up to 30 percent) than cultures of lymphocytes from other species. The ALG exerted an effect equal to that of PHA in the primary immune response (Fig. 1). In comparison with other species dog lymphocytes responded poorly to PHA. This cannot be attributed to technique or to the PHA used since over 90 percent of the cells observed in human cultures incubated with the same lot of PHA were blast cells. The addition of normal horse globulin to the dog lymphocytes resulted in the same number of blast forms as in the control cultures.

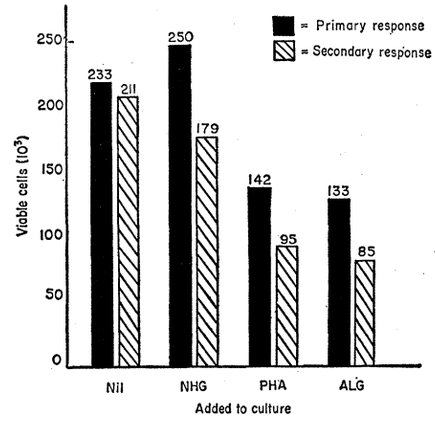


Fig. 2. Number of viable cells in lymphocyte cultures. Mean values from the primary response in eight dogs and from the secondary response in four dogs.

Viability counts showed that normal horse globulin was not cytotoxic. In comparison, PHA and ALG were cytotoxic; the number of viable cells were reduced by 36 percent and 40 percent, respectively (Fig. 2).

The reaction of dog lymphocytes in vitro therefore is quite variable and perhaps less responsive to PHA compared to results of studies on lymphocytes from other species. The increase in number of blast forms is similar to that seen with the mitogenic agent phytohemagglutinin. Our results correspond with those of Sell *et al.* (*6*) who showed that heterologous antiserum to lymphocytes was blastogenic for rabbit lymphocytes grown in culture. However, in their study ALS was less of a stimulant to lymphocytes than PHA was. Although this effect may be related to the action of ALG as a foreign protein, other mechanisms probably are active, since normal horse globulin did not cause an increase in number of blast forms. This effect in vitro appears to be similar to that observed in spleens and lymph nodes of patients treated with ALG (*3*). Furthermore, studies of viability in vitro confirmed the lymphocytotoxic effect reported by Monaco *et al.* (*1*).

Thus, lymphocyte transformation was stimulated in vitro by antiserum to lymphocytes and correlated with lymphocytotoxic effects of this antiserum. The results obtained have demonstrated the importance of determining transformation and viability studies simultaneously.

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### References and Notes

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