IMMUNOGLOBULIN PRODUCTIVITY OF HORSE LYMPHOCYTES AND ISOLATION OF ITS PROMOTING FACTOR FROM HORSE SERUM

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The capacity of immunoglobulin production in horse lymphocytes was studied by a protein A-hemolytic plaque assay, using pokeweed mitogen as a B-cell activator. In addition, the effect of horse serum on the capacity of the cells in the assay system was also examined. The results were as follows. First, the number of plaque-forming cells (PFC), immunoglobulin-producing cells, was $2,120 \pm 426$ (mean $\pm$ S.D.) per 10^6 peripheral blood mononuclear cells in five adult (6–23 years old) horses, while it was $1,512 \pm 285$ in three young (2 years old) horses. In addition, it was only 20 in one newborn horse. These results suggested that young horses, under 2 years old, were less capable of producing an immune response than adult horses.

Second, addition of horse serum resulted in a marked increase of PFC in the assay system. Therefore, we tried to find the PFC-promoting factor in horse serum, and succeeded in isolating it. The factor was a protein with an apparent molecular mass of about 80,000, composing the serum β-globulin. On the basis of analysis of its N-terminal amino acid sequence and its amino acid composition, this factor was identified to be serum transferrin (Tf). Furthermore, it was demonstrated that iron-saturated transferrin (holo-Tf) purified from horse serum accelerated plaque formation, while iron-free transferrin (apo-Tf) had no effect on the increase of PFC. This result strongly suggested that iron-binding Tf or iron *per se* was intimately connected with the ability of lymphocytes to produce immunoglobulin.

In addition, Tf was separated into three fractions on DEAE-Sephacel chromatography. Each of these three fractions accelerated the immunoglobulin production of lymphocytes.