Brief Definitive Report

ANTIPOLYSACCHARIDE ANTIBODIES OF RESTRICTED HETEROGENEITY SECRETED BY A SINGLE LYMPH NODE

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Immune responses which give rise to the synthesis of antibodies of restricted heterogeneity can be reproducibly induced in rabbits and mice by streptococcal polysaccharide antigens (1, 2). While these reports have demonstrated clonotype restriction in the immune serum of rabbits and mice, they give little information with respect to the clonotype restriction in a single reaction site of organized lymphoid tissue, for example a single lymph node. The kinetics of immune responses in lymph nodes in situ have been studied in sheep using a variety of antigens (3–6), and a substantial body of information is available on the changes in cell output, cell type, and the number of antibody-secreting cells which occur within the efferent lymph of antigen-stimulated lymph nodes. However, there is no information with respect to the amount and the heterogeneity of antibody that is secreted by the lymph node or cells within the efferent lymph.

In the present report we have examined the temporal sequence of clonal restriction in the efferent lymph of individual sheep popliteal lymph nodes undergoing an immune response to the streptococcal group A-variant polysaccharide (Av-CHO).

Materials and Methods

Experimental Protocol. The surgical procedures used for the cannulation of afferent and efferent ducts of popliteal lymph nodes were those described by Hall and Morris (7). After a postoperation period of 2 days, group A-variant streptococcal vaccine (strain A486 variant of the collection of Dr. R. C. Lancefield, The Rockefeller University, New York) containing 2.5 μg of the Av-CHO in 0.1 ml saline was infused into the lymph node via an afferent lymphatic vessel. Efferent lymph was collected 8 h before infusion of antigen and thereafter at 6–18-h intervals throughout the entire experiment.

Cell Output and Plaque-Forming Cell (PFC) Response. The number of cells in any given lymph sample was determined using a calibrated Model FN Coulter counter with a 100 μm aperture. The cell count is expressed as cell output per hour (Fig. 1).

Sheep red blood cells were coated with the *O*-stearoyl ester of the isolated, chemically pure Av-CHO, as described by Read and Braun (8). The coated red cells were used to detect PFC in Cunningham slide chambers (9).

Antibody Output. The amount of Av-CHO-specific antibody secreted into the efferent lymph was measured by a modified Farr assay (10, 11) which employed ^{125}I-labeled Av-CHO (12). In addition, the molecular restriction of IgG antibodies was determined by analytical isoelectric focusing (IEF) in polyacrylamide gels (13) using ^{131}I-labeled Av-CHO for specific binding (12). The polyacrylamide gels were subsequently exposed to photographic films to determine the position of Av-CHO-specific antibodies, as described previously (12).

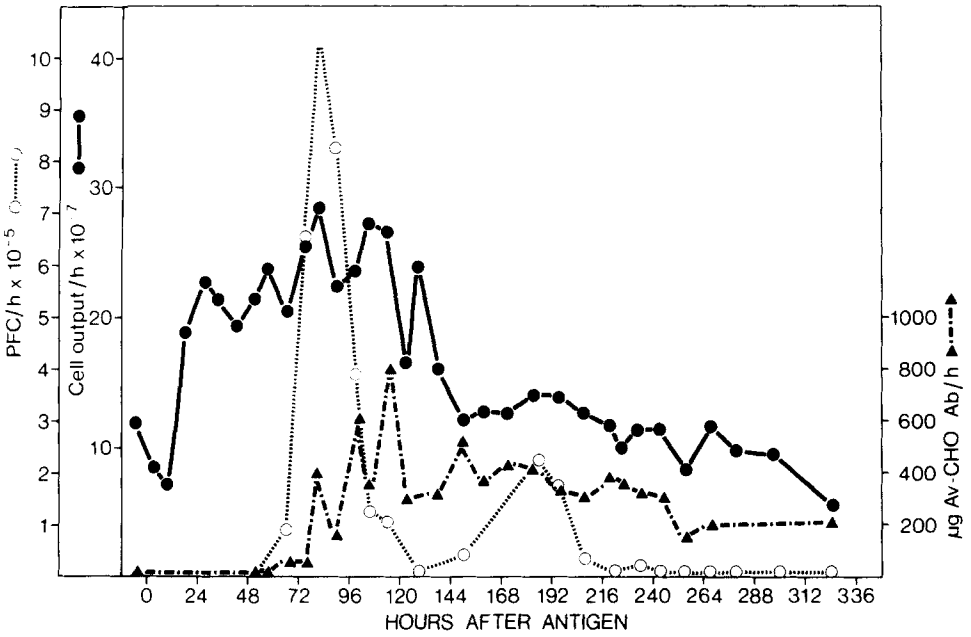

FIG. 1. The output of lymphoid cells, Av-CHO-specific PFC, and anti-Av-CHO antibody from a cannulated sheep popliteal lymph node (Sheep 182) after infusion into the afferent lymphatic vessel of A486 variant streptococcal vaccine containing 2.5 μg of Av-CHO.

Results and Discussion

Popliteal lymph nodes of three sheep were cannulated, and they were stimulated by a streptococcal group A-variant vaccine. In the following, a detailed description of one such typical experiment obtained with one sheep is given; the data are summarized in Figs. 1 and 2. Stimulation with this bacterial vaccine led to changes in cell output similar to those observed with other antigens employed in this experimental model (4–7). PFC specific for the Av-CHO first appeared 60 h after the infusion of antigen and reached a peak output in the efferent lymph some 20 h later; it coincided with the peak of total cell output in the efferent lymph. Around 180 h after the infusion of antigen a second minor peak of Av-CHO-specific PFC was observed in the efferent lymph. From 60 to 120 h after antigen was given, 25×10^6 PFC were collected in efferent lymph and 9×10^6 were collected from 120 to 264 h. After 264 h the output of PFC was in the order of 2,000 PFC per hour.

Av-CHO-specific antibodies were first detected in the efferent lymph, 60–72 h after antigen had been infused. The peak antibody secretion of 800 μg/h (110–120 h) was preceded by the peak output of Av-CHO-specific PFC (80 h). The level of Av-CHO-specific antibody in the efferent lymph then gradually decreased to 300–400 μg/h and at the conclusion of the experiment (336 h) had fallen to 200 μg/h (Fig. 1). During the experimental period of 336 h the popliteal lymph node of sheep No. 182 had secreted a total of 83 mg Av-CHO-specific antibody into the efferent lymph. Inasmuch as the peak secretion of antibody was preceded by the first peak of PFC a large proportion of the antibody must have been synthesized and secreted by cells remaining in the lymph node.

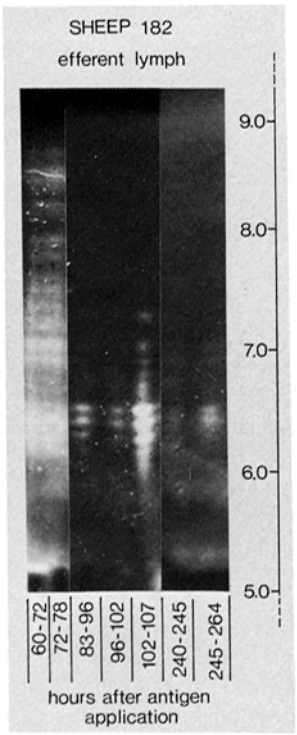

FIG. 2. IEF of cell-free lymph (30 μl) at various times after the intranodal infusion of A 486 variant streptococcal vaccine. Exposure to Agfa X-ray film revealed the areas to which ^{131}I-labeled Av-CHO has bound to specific antibody.

Further analysis of the Av-CHO-specific antibody appearing in the efferent lymph after infusion of the group A-variant vaccine considered the class of immunoglobulins secreted and the degree of heterogeneity at the clonotype level. Analysis of the lymph by radioimmunoelectrophoresis and ultracentrifugation revealed an early simultaneous secretion of specific IgM and IgG antibody during the peak output of Av-CHO-specific PFC. This IgG fraction occurring between 60–78 h postinfusion of vaccine consisted of polyclonal Av-CHO binding antibody (Fig. 2); it was subsequently replaced (at 83–96 h) by highly restricted Av-CHO-specific IgG antibody containing one predominant clonotype (pH 6.2–6.5) among two or three additional clonotypes. It was this restricted antibody pattern which persisted for the rest of the experimental period in roughly equal proportions of the clonotypes to one another.

The following conclusions can be drawn from the results of the experiments reported here:

(a) The specific Av-CHO PFC output in efferent lymph after stimulation of single lymph nodes with streptococcal A-variant vaccine follows a biphasic pattern with a major peak of activity occurring at around 80 h and a minor peak at around 180 h after the infusion of antigen. Such a pattern is similar to that induced by lipopolysaccharides in mouse spleen cells (14) and induced by aggregated heterologous IgG in rabbit spleen cells (15).

(b) Specific antibody first appears in the efferent lymph at 60–72 h after the infusion of antigen into the lymph node. PFC occur at around the same time, although the peak output of specific antibody (800 μg/h) does not coincide with the peak output of PFC. A single lymph node may secrete as much as 83 mg of specific antibody within 336 h after stimulation with 2.5 μg of Av–CHO presented on whole heat-killed bacteria.

(c) After stimulation the initial secretion of antibodies into the lymph involves low levels of heterogeneous IgG antibodies and IgM antibodies and is followed by clonally restricted IgG antibodies which dominate the response. Further work will deal with this mode of selection, a selection that is in accordance with observations in rabbit and mouse models (16, 17), but opposite to that which has been reported for anti-DNP responses (18).

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