THE ANTIGEN BINDING DENDRITIC CELL OF THE LYMPHOID FOLLICLES: EVIDENCE INDICATING ITS ROLE IN THE MAINTENANCE AND REGULATION OF SERUM ANTIBODY LEVELS

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INTRODUCTION

Antigen (Ag) is obligatory for the induction of immune responses. It has been postulated that Ag continues to play a role in their maintenance and regulation, however, the evidence for such a role is largely circumstantial (1-5). In fact, the unequivocal demonstration of a readily degradable Ag persisting in the presence of immune clearance mechanisms has not been reported. The present investigation was undertaken to see how long a simple protein Ag could persist in an immune animal and to see whether retained Ag might be implicated in the maintenance and regulation of antibody (Ab) production.

MATERIALS AND METHODS

Solubilization of the $^{125\text{I}}$ labeled antigen from lymph nodes. The popliteal lymph nodes were removed, teased apart in phosphate buffered saline (PBS) containing 5M guanidine hydrochloride and 100 µg/ml cold Human Serum Albumin (HSA). This mixture was incubated in a 37°C H$_2$O bath for 30 min and then dialized against PBS. After centrifugation, 80 to 90% of the radiolabel originally present in the lymph node was in the supernatant.

Coprecipitation. Sufficient rabbit anti-HSA was added to the solubilized HSA to establish equivalence and the mixture was allowed

1 This work was carried out while on leave from the Department of Microbiology, Medical College of Virginia, P.O. Box 847, Richmond, Virginia 23298, USA.
TAB. 1: The Kinetics of Antigen Clearance from the Popliteal Lymph Nodes of Immunized and Non-Immunized Mice.

<table>
<thead>
<tr>
<th>Immune Status</th>
<th>Picograms $^{125}$I-HSA per mg of tissue (mean ± SE)</th>
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<tbody>
<tr>
<td></td>
<td>day 0.2</td>
</tr>
<tr>
<td>Immunized</td>
<td>1,720±660</td>
</tr>
<tr>
<td>Non-Immunized</td>
<td>820±50</td>
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</tbody>
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(a) The popliteal lymph nodes from immune mice were much larger than the nodes from non-immune mice. The data are therefore expressed as picograms $^{125}$I-HSA per mg of lymph node.

to incubate overnight at 4°C. The precipitate was collected by centrifugation, washed twice, and the radioactivity was counted.

Other methods. Techniques for iodination of proteins, immunization of mice, autoradiography, cell culture, and the radioimmunnoassay for mouse anti-HSA, have been described previously (6).

RESULTS

The kinetics of clearance of $^{125}$I labeled HSA from the popliteal lymph nodes of immune and non-immune CBA/H WEHI mice was studied (Tab.1). For the first 3 days radiolabel was cleared rapidly from both groups of animals. However, by day 6 a difference between the immune and non-immune groups was apparent. Less radioactivity was present in the nodes from non-immune animals and this level was declining rapidly. In contrast, radioactivity in the nodes from immune animals declined slowly. In a separate experiment the half-life of retained Ag was 2 mo (95% confidence interval between 1.3 and 5 mo) in the period between 10 and 45 days.

Autoradiography revealed that 4 hours after injection, heavy label was around germinal centers and over macrophages of the medulla and superficial cortex. The same pattern was present at 24 hours except there were very few grains left in macrophages. By the 3rd day, long exposure times were required to detect radioactivity in macrophages, but the labelling remained heavy in lymphoid follicles. Electron microscopy in conjunction with autoradiography indicated that retained radiolabel was associated with processes of dendritic cells. To examine the rate of Ag catabolism in macrophages more carefully, peritoneal macrophages were allowed to engulf radiolabelled