CODISTRIBUTION OF ANTIGEN AND Fab DETERMINANTS ON THYMIC ANTIGEN-BINDING CELLS

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INTRODUCTION

In spite of the fact that thymus-derived lymphocytes (T cells) bind antigens specifically (1), interaction between the surface receptor and antigen on these cells has not been directly visualized as it has been for bone marrow-derived lymphocytes (B cells) (2-4). Several investigators have recently established that avian antibodies specific for mammalian Ig bind to T cells, as well as to B cells, and that this binding is demonstrable by immunofluorescence (5-8). If the T cell surface components visualized by avian anti-mammalian Ig are involved in primary immune recognition, these components should be coincidentally distributed with antigen on the surface of ABL from thymus.

In this communication, we present results of studies designed to test this question. Our experiments involved the simultaneous localization of Ig and antigen using two immunofluorescence labels, fluorescein and rhodamine. Our studies were performed using thymus

Abbreviations used in this paper: ABL, antigen-binding lymphocyte(s); CAM(Fab')2, chicken anti-mouse (Fab')2 fragments; DMSO, dimethyl sulfoxide; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; HBSS, Hanks balanced salt solution; HSF, horse spleen ferritin; Ig, immunoglobulin(s); KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; NCG, normal chicken globulin; PBS, phosphate buffered saline; RACG, rabbit anti-chicken globulin; RAMG, rabbit anti-mouse globulin; TRITC, tetramethyl-rhodamine isothiocyanate; NMtG, normal mouse gamma globulin.
TAB. 1: Frequency of Antigen-binding Cells in Thymus and Spleen of Mice

<table>
<thead>
<tr>
<th>Organ</th>
<th>KLH</th>
<th>HSF</th>
<th>MYO</th>
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</thead>
<tbody>
<tr>
<td>Thymus (BALB/c)</td>
<td>13 ± 4</td>
<td>8 ± 3</td>
<td>8 ± 4</td>
</tr>
<tr>
<td>Thymus (C3H)</td>
<td>5</td>
<td>2 ± 0</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Spleen (BALB/c)</td>
<td>26</td>
<td>16</td>
<td>22 ± 1</td>
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Lymphocytes from unimmunized mice and specifically purified chicken antibodies raised against the (Fab')$_2$ fragment of IgG isolated from normal mouse sera (6,7). Additional studies with anti-Thy-1.2 and anti-H-2$^d$ sera were performed to demonstrate the T cell nature of ABL, and to provide contrasting patterns of distribution of antigen and non-Ig surface markers.

MATERIALS AND METHODS

Five µl of rhodamine-labeled antigen (in 5 x 10$^{-2}$ M NaN$_3$) was added to 50 µl of a 95% viable cell suspension from BALB/c or C3H mice (giving a final antigen concentration of approximately 500 µg/ml for about 10$^7$ total cells and 5 x 10$^{-3}$ M NaN$_3$) for 1 h at 0°C in HBSS + 5% FCS. Then, a 1:20 dilution of anti-BALB/c serum, 1:20 anti-Thy-1.2 serum, or 1:10 CAM(Fab')$_2$ was added to the cells while in the presence of antigen for an additional hour at 0°C. After two washes with either 5 ml of HBSS plus FCS, or with 1 ml of 100% FCS, the cells were resuspended in 100 µl HBSS plus 5% FCS and incubated with a 1:20 dilution of the appropriate developing reagent [RAGG (FITC) or RAMG(FITC)] for another hour at 0°C before a final washing was done as before. Cells were then fixed in 1% p-formaldehyde in 0.1 M cacodylate buffer, pH 7.0. In order to slow cell motion, a drop of pelleted cell suspension was mixed with a drop of 90% glycerin in PBS on a glass slide, before being covered with a coverslip.

Observation, counting and photography of ABL were accomplished with a Zeiss photomicroscope III equipped with a fluorescence epi-