CHAPTER 15
CELL INTERACTIONS IN HUMORAL RESPONSES:
THE CARRIER EFFECT

A primary humoral immune response requires the cooperation of three cell types, an ANTIGEN-PRESENTING CELL (APC, typically a dendritic cell or macrophage), a T-HELPER CELL (T\textsubscript{H2}), and a B-CELL. The APC/T-CELL interaction involves the recognition of MHC Class II-bound peptide by the T-cell receptor, and the participation of the accessory molecule CD4. The same principles apply to secondary humoral responses, except that the B-cell can also take on the role of APC, presenting processed antigen to memory T-cells; thus, only two cells (B-cell and memory T-cell) need participate in this interaction. This two-cell interaction is the basis for understanding the experimental phenomenon of the CARRIER EFFECT.

"THREE-CELL INTERACTION" IN IMMUNE RESPONSES

The generation of immune responses generally requires the participation of three classes of lymphoid cells, an antigen-presenting cell (APC), a helper cell and an effector cell. We have seen one example of this in the generation of cell-mediated immunity outlined in Chapter 12; in that case the APC was a macrophage or dendritic cell, the helper was a T\textsubscript{H1} cell, and the effector was a T\textsubscript{C} cell.

The same principle holds in the cell interactions required for the development of humoral responses. At the top of Figure 15-1 is shown such an interaction, involving an APC, a T\textsubscript{H2} cell (a virgin T\textsubscript{H2} cell, as opposed to a memory cell, discussed further below), and a B-cell. This interaction is similar in most respects to the one we have already seen in Chapter 12. The APC bears surface antigen associated with Class II MHC molecules which is recognized by the T-cell; the T-cell receptor provides the antigen-specific recognition, and the CD4/Class II contact stabilizes this interaction. The antigen-presenting cell provides a "costimulatory" signal to the T-cell in the form of its membrane B7 molecule (which is recognized and bound by CD28 on the T\textsubscript{H2} cell) and the T\textsubscript{H} cell then responds by secreting IL-4. The cell which responds to this IL-4 is a nearby B-cell, which bears membrane Ig receptors (specific for LCMV) which have already been occupied and cross-linked by antigen; this B-cell responds to IL-4 by differentiating into an antibody-forming cell and undergoing clonal expansion.

Memory T\textsubscript{H}-cells (T\textsubscript{H(M)}-cells) can participate in a second kind of interaction, illustrated at the bottom of Figure 15-1. This two-cell interaction results from the fact that B-cells are capable of acting as efficient APC's for memory T\textsubscript{H} cells. (It’s worth noting that we might have predicted this, based on the fact that B-cells constitutively bear Class II molecules on their surface.) In this interaction, the B-cell plays two roles. First, it acts as an APC, by presenting Class-II-associated viral antigen to the T\textsubscript{H(M)}-cell and expressing the costimulatory molecule B7. Second, it then acts as an effector cell by responding to the IL-4 secreted by the T-cell, and it undergoes differentiation and clonal expansion. Thus, this interaction can still be conceptually regarded as a "three-cell" interaction (APC/Helper/Effector), despite the fact that only two distinct cells participate.
Figure 15-1

CELL INTERACTIONS IN HUMORAL IMMUNITY
T/B-CELL COOPERATION

The special importance of B-cells as APCs for memory T-cells stems from the fact that they are clonally precommitted; those rare memory B-cells (perhaps one in a thousand) which happen to be specific for an epitope on a particular antigen can very effectively bind that antigen by their membrane Ig receptors. Having bound the antigen, the B-cell then internalizes it (by receptor-mediated endocytosis, not phagocytosis), associates its degraded peptides with newly synthesized Class II MHC molecules, and displays these new class II molecules on its surface. Those memory T\textsubscript{H} cells which happen to be clonally precommitted to carrier epitopes on the antigen (also rare cells) can recognize the Class II-associated carrier determinants via their T-cell receptors. (Why such B-cell-dependent antigen presentation plays only a small role in primary responses is not immediately obvious, since B-cells' advantages as APCs should in principle still hold; it may be that prior to clonal expansion and affinity maturation they are too rare or bind antigen too poorly to play a more effective role.)

The signaling which then follows is the same as we have already seen in CMI, namely the transmission of a co-stimulatory signal by the APC (B-cell), which triggers the helper T cell to produce IL-4, which then in turn stimulates the B-cell (which bears IL-4 receptors). Thus, two signals are necessary for triggering a B-cell to differentiate into an effector (=Ab-secreting) cell: first, its antigen-specific Ig receptors must be occupied and cross-linked; second, it must receive appropriate signals (e.g. IL-4) from a helper cell. As we have seen, the requirement for two signals is analogous to that of both T\textsubscript{H1} and T\textsubscript{C} cells in CMI.

For antibody production to T-dependent antigens, T-cell/B-cell interaction is required for both primary and secondary responses although the nature of the interactions appears to differ in the two cases (as we have seen in Figure 15-1). We will illustrate some of the consequences of this model as applied to secondary humoral responses by two experiments which, more than 40 years ago, defined the CARRIER EFFECT.

EXPERIMENTAL ANALYSIS OF THE CARRIER EFFECT

The key features of the T-cell/B-cell interaction for humoral immune responses, which we will illustrate using the Carrier Effect, are as follows:

1) Two classes of lymphocyte are required, Th-cells and B-cells.

2) T and B-cells recognize different epitopes, or “antigenic determinants”, on the same antigen molecule. Those recognized by B-cells we call hapten determinants, those recognized by T-cells are carrier determinants.

We can illustrate these requirements with two simple experiments in mice, using the DNP (dinitrophenyl) group as a hapten, and three unrelated (i.e. non-cross-reactive) proteins as carriers, namely bovine gamma globulin [BGG], bovine serum albumin [BSA] and chicken ovalbumin [OVA].

In order to understand these experiments, it’s important to keep in mind two basic features.
1. **We are measuring only antibody (humoral) responses to the hapten DNP.** Humoral immune responses against the carrier will also take place, but we will ignore them. Cell-mediated responses against the carrier may also be generated, but we will not concern ourselves with these either.

2. **We are measuring only secondary, memory responses**, which result in *high levels of IgG antibody*. Primary humoral responses to DNP will certainly be generated, but these will produce only low levels of IgM and, again, we will ignore them.

As will be illustrated in the experiments below, **generation of these secondary responses requires the presence of both memory B-cells and memory T-cells**, each of which must have been generated by a previous primary immunization.

**EXPERIMENT 1: CARRIER EFFECT**

The host must be primed to both hapten and carrier to generate a response, and must be challenged with both on the same molecule.

We immunize ("prime") mice with various antigens, then challenge ("boost") two weeks later. We then test their serum for the presence of anti-DNP antibodies one week after the boost.

<table>
<thead>
<tr>
<th>Prime</th>
<th>Challenge</th>
<th>Test for anti-DNP Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) DNP alone</td>
<td>DNP-BGG</td>
<td>-</td>
</tr>
<tr>
<td>2) DNP-BGG</td>
<td>DNP-BGG</td>
<td>+</td>
</tr>
<tr>
<td>3) DNP-BGG</td>
<td>DNP-BSA</td>
<td>-</td>
</tr>
<tr>
<td>4) DNP-BGG</td>
<td>BGG</td>
<td>-</td>
</tr>
<tr>
<td>5) DNP-BGG</td>
<td>DNP-BSA + BGG</td>
<td>-</td>
</tr>
</tbody>
</table>

As can be seen in line 3, priming with DNP-BGG will *not* result in an anti-DNP response to a boost with DNP on *another* carrier (BSA). This defines the **carrier effect**: the host’s immune status toward the carrier affects the response to the hapten.

**EXPERIMENT 2: SEPARATE CARRIER AND HAPTEN PRIMING**

The host must have been primed to *both the carrier and the hapten* used for challenge; however, the priming can be carried out separately for the two kinds of epitopes.

We will give the mice *two* primary immunizations; these can be given simultaneously (in two different sites) or a week apart. We will then continue as before, challenging after two weeks then testing for the presence of anti-DNP antibody one week later.
### Primary Immunizations

<table>
<thead>
<tr>
<th>#1</th>
<th>#2</th>
<th>Challenge</th>
<th>Anti-DNP Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) DNP-OVA</td>
<td>–</td>
<td>DNP-OVA</td>
<td>+</td>
</tr>
<tr>
<td>2) DNP-OVA</td>
<td>–</td>
<td>DNP-BGG</td>
<td>–</td>
</tr>
<tr>
<td>3) DNP-OVA</td>
<td>BGG</td>
<td>DNP-BGG</td>
<td>+</td>
</tr>
<tr>
<td>4) DNP-BGG</td>
<td>OVA</td>
<td>OVA</td>
<td>–</td>
</tr>
<tr>
<td>5) DNP-BGG</td>
<td>OVA</td>
<td>DNP-OVA</td>
<td>+</td>
</tr>
<tr>
<td>etc.</td>
<td></td>
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</tbody>
</table>

In order to produce a secondary anti-DNP response after challenge with DNP-BGG, the animal must have been primed to both DNP and BGG (line 2); however, as seen in line 3, the animal can be separately immunized to DNP (on any carrier) and BGG (without DNP). Therefore, the recognition of hapten and carrier determinants must be executed by separate cell populations, since they can be separately primed.

By carrying out additional experiments not shown here, one can demonstrate that those primed cells required for recognition of the carrier are T-cells (i.e. they can be killed with antibodies to the mouse T-cell-specific antigen Thy-1), while those cells recognizing the hapten are B-cells (not affected by anti Thy-1 treatment).

The basic feature of T-cell B-cell cooperation that is demonstrated here is that two different cells need to recognize different antigenic specificities on the immunogen in order to cooperate. The names "hapten" and "carrier" in this context refer simply to those epitopes recognized by B-cells and T-cells, respectively, but some clarification may be useful. The epitope recognized by the B-cells does not necessarily have to be a "hapten" in the strict sense of the term (i.e. a small chemical moiety which can be coupled to different carriers). Any suitable target, including portions of a "carrier" protein molecule, could be recognized by B-cells and serve as the "hapten" portion of the hapten/carrier phenomenon; the use of a "moveable" classic hapten such as DNP simply makes the design and interpretation of such experiments much easier.

The "carrier" specificity, however, has more limited possibilities. In theory, T-cells could use DNP as a "carrier" specificity and could then cooperate with B-cells for an antibody response to some other (or the same) "hapten" determinant present on the BSA molecule. However, DNP and other typical haptons cannot be recognized by T-cells, because they are not peptides and therefore cannot bind to the peptide-binding groove of an MHC molecule (see Chapter 12). Thus, while B-cells using their cell surface immunoglobulin can bind to both peptide antigens, either free or within a larger protein, and haptons such as DNP, T-cells, which use their TCR for antigen recognition, are largely restricted to recognizing only peptides intimately associated with a suitable MHC molecule.

**TWO KINDS OF T-INDEPENDENT ANTIGENS: TI-1 AND TI-2**

The cell interactions shown in Figure 15-1 and illustrated by the Carrier Effect are required for humoral responses to T-dependent (TD) antigens (see Chapter 13). As already mentioned, however, there are some antigens which do not require T-cell help to generate a humoral
immune response, and these are known as *T-independent (TI)* antigens. Two general classes of T-independent antigens can be distinguished, which have been named *TI-1* and *TI-2*.

TI-1 antigens are exemplified by *lipopolysaccharide* (LPS), a component of the cell wall of Gram-negative bacteria. LPS is mitogenic for B-cells, and can therefore efficiently activate any B-cell with LPS-binding antigen receptors, even if the LPS is present only at very low concentrations, which results in an antibody response. In effect, the mitogenic activity of LPS replaces the need for the “second signal” normally provided by the T\(H\) cell.

TI-2 antigens, on the other hand, are not mitogenic, but typically bear highly repetitive epitopes (for example, high molecular weight carbohydrates). The exceptionally high degree of Ig-receptor cross-linking produced by such antigens seems to provide a very high level of “Signal 1”, and reduces or eliminates altogether the requirement for “Signal 2” (normally provided by IL-4 and other lymphokines). However, in the absence of antigen-specific T-cell help, humoral immune responses to TI antigens of either type generally do not exhibit class switching, and are therefore largely restricted to triggering IgM responses (as, for example, the ABO blood group antigens discussed in Chapter 10).

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**CHAPTER 15, STUDY QUESTIONS:**

1. Describe the different cell/cell interactions involved in PRIMARY versus SECONDARY humoral responses.

2. What are the critical cellular requirements for generating an IgG ANTI-HAP TEN HUMORAL RESPONSE in response to the injection of DNP coupled to a carrier?

3. Define and distinguish T\(H_1\) and T\(H_2\) cells.

4. Define and describe the two classes of *T-independent* antigens.