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TWO TYPES OF MURINE HELPER T CELL CLONE

1. Definition According to Profiles of Lymphokine Activities and Secreted Proteins

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A panel of antigen-specific mouse helper T cell clones was characterized according to patterns of lymphokine activity production, and two types of T cell were distinguished. Type 1 T helper cells (Th1) produced IL-2, interferon-γ, GM-CSF, and IL-3 in response to antigen + presenting cells or to Con A, whereas type 2 helper T cells (Th2) produced IL-3, Bsf1, and two other activities unique to the Th2 subset, a mast cell growth factor distinct from IL-3 and a T cell growth factor distinct from IL-2. Clones representing each type of T cell were characterized, and the pattern of lymphokine activities was consistent within each set. The secreted proteins induced by Con A were analyzed by biosynthetic labeling and SDS gel electrophoresis, and significant differences were seen between the two groups of T cell line. Both types of T cell grew in response to alternating cycles of antigen stimulation, followed by growth in IL-2-containing medium. Examples of both types of T cell were also specific for or restricted by the I region of the MHC, and the surface marker phenotype of the majority of both types was Ly-1+, Lyt-2-, L3T4+. Both types of helper T cell could provide help for B cells, but the nature of the help differed. Th1 cells were found among examples of T cell clones specific for chicken RBC and mouse alloantigens. Th2 cells were found among clones specific for mouse alloantigens, fowl γ-globulin, and KLH. The relationship between these two types of T cells and previously described subsets of T helper cells is discussed.

Mouse helper T cells (Th1) appear to be fairly uniform when judged by the commonly used surface markers bearing Ly-1 and L3T4 but not Lyt-2 and L3T4. When assessed by functional criteria, however, at least two types of Th1 cell have been described (1–6). These subtypes of Th1 cell have been described mainly in uncloned populations, but certain differences between them appear to be consistent. A synthesis of the work of several groups provides the following description of these T cells. One type of Th1 cell (Th1) helps B cells in a linked, antigen-specific manner, is "monogamous" in limiting dilution cultures, and is required early in the response. These T cells may also help in an unlinked manner at high antigen concentrations (7). The other type of Th1 cell (Th2) helps B cells in a nonlinked manner, is "polymonogamous" in limiting dilution cultures, and is required later in the response. In addition, Th2 cells possess determinants that map to the I region of the major histocompatibility (MHC) and bind to nylon wool, whereas Th2 cells lack both of these properties. The determinants on Th2 cells that map to the I region may actually represent structures that are controlled by the I region but are not coded by the I region (8).

We have found that in a large panel of antigen-specific and autoreactive Th1 cell clones, we were able to divide the clones into two distinct groups. These groups were originally defined on the basis of patterns of lymphokine synthesis, but can also be distinguished by their characteristic mode of B cell help. The properties of these two sets of T cells agree in many respects with the two sets of Th1 cells described above, and we have used the nomenclature of Tada et al. [1] to describe Th1 and Th2 cells. In this study we describe the derivation of antigen-specific T cell clones of both types, and their evaluation according to the distinctive patterns of lymphokine activities produced after antigen or lectin stimulation. We have also analyzed the supernatant proteins produced after concanavalin A (Con A) stimulation and biosynthetic labeling, and have found characteristic patterns associated with each type of Th1 cell clone.

MATERIALS AND METHODS

Mice. C57BL/6, CB6F1, and BALB/c mice were obtained from THE Jackson Laboratory (Bar Harbor, ME) and the Institute for Medical Research (San Jose, CA). Recombinant inbred mice used for MHC restriction typing were a gift from Dr. H. McDevitt. Antigens and antibodies. Chicken red blood cells (CRBC) were obtained from six partially inbred lines of chickens, two monoclonals for MHC alleles but differing in other blood group antigens, maintained at PMK Farms, Vacaville, CA. Keyhole limpet hemocyanin (KLH) was obtained from Pacific Bionaire, Venice, CA, and fowl γ-globulin (FGG) was obtained from Cappel Laboratories, Cochranville, PA. Monoclonal antibody specific for mouse interleukin 2 (IL-2) was from the S486 hybridoma obtained by fusing Sp2/0 hybridoma cells with spleen cells from a rat immunized with concentrated LD51-1 supernatant [in preparation].

Cell lines. The COS monkey cell line was obtained from F. Lee. The HT2 mouse T cell line (9) was obtained from S. Strober, the
Two types of cloned helper T cell

NFS60 cell line was obtained from J. Ihle through D. Rennick, and the MC/9 mast cell line (10) and C1-Ly-1/9 T cell line (11) were obtained from G. Nabel. The MM3 mouse mast cell line was established from the bone marrow of a DBA/2 mice immunized with Plasmodium berghei sporozoites (12, 13) and was maintained in RPMI 1640 supplemented with 10% FBS and 20 ng/ml of IL-3. The MM3-LacZ cell line was established from MM3 cells infected with a murine retrovirus expressing the lacZ gene under the control of the murine I-A 

biosynthetic labeling and immune precipitation. T cells were stimulated with Con A and were labeled with [3H]methionine as described. Supernatants containing cytokines were concentrated by using either normal rabbit serum or a rabbit anti-mouse interferon-γ antisem (Enzo Biochem, New York, NY) by described methods.

RESULTS

Characteristics of bioassays. A large panel of antigen-specific T cell lines was analyzed by using several lymphokine bioassays. The cell lines could be grouped into two sets. T1 and T2, on the basis of the characteristic pattern of bioactivities found in their supernatants after Con A or antigen stimulation. We used several target cell lines in bioassays to detect these lymphokine activities. Some of these assays detect more than one factor, and some activities are influenced by the presence or absence of other lymphokines. We have characterized some of these interactions on three target cell lines to use the bioassays as more precise tools for determining the presence or absence of certain lymphokines. In particular, we have established “dominant” assays, i.e., bioassays in which a lymphokine activity is not influenced by the presence of T1 or T2 supernatants. In the description of the bioassays that follows, we have used the L929-T1 cell clone and the representative T1 cell lines MB1-1 T1 and MB2-1 as the T2 cell.

HT2 assay. The mouse T cell line HT2, originally established by J. Watson (9), is commonly used as an assay for mouse and human IL-2. We have used this line for IL-2 assays, and have also used it to assay another lymphokine activity in supernatants of certain T cell clones. Con A does not influence the HT2 cell line at the concentrations present in the samples (results not shown). Figure 1A indicates that HT2 proliferated equally well in response to saturating amounts of either recombinant IL-2 or the Con A-induced supernatant from MB2-1 (the T cell line from which the recombinant cDNA clone for IL-2 was derived) (14). The addition of supernatant from the MB2-1 T cell line did not influence the stimulation to IL-2 derived either from recombinant sources or from IL-2 supernatant (Fig. 1B). Taken together, these results suggest that HT2 cells respond to IL-2 and that this stimulation is not influenced by other factors present in induced IL-2 or MB2-1 supernatants (examples of T1 and T2 cells, respectively). Figure 1A also demonstrates that the supernatant of induced MB2-1 cells contains an activity that stimulated the HT2 cell line to a lesser extent than IL-2, even at saturating amounts of supernatant. Similar results have been obtained by others (D. Rennick, personal communication). We have used a monoclonal antibody specific for mouse IL-2 to demonstrate that this second activity was distinct from IL-2. The S4B6 monoclonal antibody is a rat anti-mouse IL-2 that recognizes mouse but not human IL-2, and appears to have a high affinity for mouse IL-2 because it completely inhibits the biological activity of IL-2 at low antibody concentrations (manuscript in preparation). The bioactivity in the T2 supernatant was unaffected by the monoclonal antibody S4B6, whereas IL-2 activity could be completely inhibited (Fig. 1C). Thus, the cell line HT2 can be used as a dominant assay for IL-2, i.e., the response of HT2 to IL-2 is not altered by other T1 or T2 supernatant activities. In

addition, HT2 cells can also be used to detect another activity in the T_{h}2 supernatants. This second activity is obscured if IL-2 is present in the sample, because the strong IL-2 signal is dominant.

Separation of lymphokine activities. Additional evidence for the separate identities of IL-2 and T cell growth factor 2 (TCGF2), and the absence of TCGF2 in LB2-1 supernatants was obtained by column chromatography. Con A-induced supernatants from LB2-1 and MB2-1 cells were separated by chromatography on a C4 reverse phase HPLC column, and individual fractions were assayed for activity in the HT2 assay. The HT2 growth factor activities from these two cell lines eluted at very different times (Fig. 2), and each cell line produced only one activity, i.e., LB2-1 did not produce a TCGF activity eluting at the same position as the TCGF2 activity from the MB2-1 sample. Neither peak of activity could be due to Con A, which elutes at a different time. The characteristically different titration curves of IL-2 and TCGF2 from LB2-1 and MB2-1 respectively were also seen in the assay of these column fractions (results not shown).

NFs60 assay. The NFs60 cell line proliferated in response to recombinant IL-3 and also in response to both LB2-1 and MB2-1 supernatants (Fig. 3A). The level of proliferation in response to saturating amounts of LB2-1

**Figure 1.** Response of HT2 cells to IL-2 and a second TCGF activity
A. Dilutions of Con A-induced LB2-1 and MB2-1 cell supernatants and recombinant mouse IL-2 were tested for activity in the HT2 proliferation assay. The starting dilutions in the first well were: LB2-1, 1/4; MB2-1, 1/1; recombinant IL-2, 1/4000. ---] LB2-1,/board] MB2-1, (---) IL2-2. B. Dilutions of MB2-1 supernatant were tested for an effect on the proliferation of HT2 cells in response to a constant amount of IL-2 (400 U/ml) supplied as a dilution of Con A-induced LB2-1 supernatant (---) or recombinant mouse IL-2 (---). A titration curve of IL-2 in LB2-1 supernatant is shown for comparison (---). Staining dilutions were: LB2-1, 1/4; MB2-1, 1/1. C. Dilutions of ascites fluid containing the monoclonal anti-IL-2 antibody S4B6 (starting at 1/40) were assayed for their effect on the response of HT2 to a constant amount of recombinant mouse IL-2 (80 U/ml, ---) or MB2-1 supernatant (1/40 final dilution, ---).

**Figure 2.** Reverse phase HPLC of IL-2 and TCGF2. Supernatants from Con A-induced LB2-1 and MB2-1 cells were concentrated and separated on a C4 column as described in Materials and Methods. Dilutions of each fraction were then assayed on HT2 cells, and the units of activity per milliliter of the column fraction were calculated.
supernatant was significantly lower than the responses to the other two IL 3 sources. Mixtures of LB2-1 supernatant with recombinant IL 3 resulted in the inhibition of the strong IL 3 signal. Interferon-γ also inhibited the proliferation of NFS60 cells in response to IL 3 (Fig. 3b). Because LB2-1 cells produce large amounts of interferon-γ, this could explain the lower maximum proliferation level of NFS60 cells in LB2-1 supernatant. Thus, NFS60 responds strongly to IL 3, and the IL 3 response can be partially inhibited by an activity in T11 supernatants, possibly interferon-γ.

**MM3 assay.** The MM3 mast cell line proliferated strongly in response to MB2-1 supernatant and weakly in response to LB2-1 supernatant or recombinant IL 3 (Fig. 4a). When LB2-1 and MB2-1 supernatants were mixed, the higher signal was obtained (Fig. 4b). These results suggest that another activity in MB2-1 supernatants, in addition to IL 3, was responsible for the maximum proliferation of MM3 cells. This second activity has been separated biochemically from IL 3 and has been shown to require IL 3 as cofactor for biological activity (unpublished results). Similar results have been obtained by Rennick and Smith [19]. This activity (which we shall refer to as mast cell growth factor 2; MCGF2) was not inhibited by any activity in LB2-1 supernatant (Fig. 4b).

**Classification of T cell clones according to lymphokine activities.** Figure 5 shows the typical activity profiles of several T11 and T12 cell lines in the HT2, MM3, and NFS60 assays. T11 cells gave results consistent with the production of IL 2, IL 3, and interferon-γ, but not MCGF2. In contrast, T12 cells did not appear to produce IL 2 or interferon-γ but did produce IL 3 and the two activities described above as TCGF2 and MCGF2. It is important to note that these sets of activities were coordinate, e.g., IL 2 and interferon-γ activities were produced by the same lines, and TCGF2 and MCGF2 activities were found together. The lymphokines that we infer were...
in some cases to remove IL 2. IL 3 was evaluated by combining information from proliferation of NFS60 and MM3 cell lines and HPLC separations [unpublished results]. Interferon-γ was evaluated by combining data from the inhibition of NFS60 proliferation, LA induction on P388D1 cells, antiviral activity, immune precipitation (see Fig. 8),α and inhibition of IgE-enhancing activity [17]. B cell stimulating factor 1 (BSF1) activity was evaluated from LA induction assays on B cells. IgE- and IgG1-enhancing activities were determined by measuring the IgE and IgG1 levels at day 7 in LPS-stimulated cultures of T-depleted spleen cells [17].

Production of lymphokine activities in response to antigen. In the experiments described above, Con A was used to stimulate T cells to produce lymphokines without interference by spleen cells. We also assayed supernatants from T cells stimulated with antigen + irradiated spleen cells, or with MHC alloantigens. In each case, the lymphokine activities induced by antigen or by Con A were identical, i.e., the activities detected were dependent on the T cell line and not on the method of induction. Representative examples are shown in Figure 6.

Classification of T cell lines according to B cell stimulating activities. Examples of the two sets of T cell lines were also tested for their effect on LPS-stimulated B cells. Activities in T cell supernatants have recently been described that enhance IgE production by over 100-fold [17] and IgG1 production by 10-fold [17, 20] in LPS-activated B cells. When supernatants from our panel of T cell lines were tested for these activities, the Tn2 cells were found to produce IgE- and IgG1-enhancing activities, whereas Tn1 cells were consistently negative in this assay (Table II). Interferon-γ is known to be a potent inhibitor of both enhancing activities [17]. Because Tn1 cells produce large amounts of interferon-γ, the absence of these enhancing activities in the Tn1 supernatants does not prove that the factor or factors that mediate this activity are absent. Some T cell supernatants also contain an activity that stimulates the expression of la antigens on normal B cells. This activity, as well as the IgG1- and IgE-enhancing activities, may be mediated by the lymphokine BSF1 [18, 20–22]. This Tn1 and Tn2 cell supernatants were assayed for the ability to induce la on B cells. Tn2 supernatants were found to contain substantial amounts of this activity, whereas Tn1 cell supernatants did not (Table III). la induction by Tn2 supernatants is not inhibited by purified interferon-γ (R. Coffman, unpublished observations).

Classification of T cell clones according to surface antigens. Examples of the same panel of T cell clones were tested for surface markers by using monoclonal antibodies directed against Lyt-1, Lyt-2, and L3T4 antigens (Table IV). In all cases tested, both Tn1 and Tn2 cell clones were L3T4+ and Lyt-2+. The majority of both types of Tn1 cell clones were also Lyt-1+1, although two Tn2 and one Tn1 cell clones were negative for this antigen. The Lyt-1 determinant may be preferentially lost during prolonged in vitro culture, because the C1.Lyt-1/9 cell line was originally Lyt-1+ (11). Representative examples are shown in Table IV.

Specificity or restriction for MHC determinants. Several T cell clones of both types were tested for MHC restriction or specificity by stimulation with spleen cells from recombinant inbred mice of the B10 series. The

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Figure 5. Bioassay of supernatants from Con A-stimulated T cell clones. Four Tn1 and two Tn2 cell lines were stimulated with Con A and the resulting supernatants were assayed on HT2, NFS60, and MM3 cell lines. The MM3 assay medium was supplemented with 2% Con A-induced L92-1 supernatant. All starting dilutions were 1/4. L92-1 , MD13-10 , MD13-10 , MD13-10 , MD13-10 , MD13-10 .

Produced by these two sets of T cell lines are summarized in Table I. IL 2 was evaluated by stimulation of HT2 cells, and MCGF2 was measured by the stimulation of MM3 cells in the presence of saturating amounts of IL 3. TCGF2 was evaluated by its weak stimulation of HT2 cells in the absence of IL 2 by using separation by HPLC.
## Two Types of Cloned Helper T Cell

### Table 1

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* Lymphokine activities were evaluated by assays described in the text.
* CRC: MHC alleles 2, 14, 15, 19, and 21, but not 13.
* CRC: all MHC alleles.
* CRBC: MHC alleles 2, 13, 14, 15, and 19, but not 21.
* Not determined.
* Not applicable.

Results in Table V demonstrate that the MHC restriction of the T cell clones specific for foreign antigens was consistent with determinations coded by the I region of the MHC, and the T cells specific for alloantigens also recognized determinants that mapped to the I region.

Secreted proteins synthesized by Tα1 and Tα2 cells. Several Tα1 and Tα2 cell lines were labeled with [35S] methionine after Con A stimulation, and the resulting induced secreted proteins were analyzed by SDS-PAGE and fluorography. We have shown previously that under these conditions, T cell clones secrete large amounts of proteins in the 10,000 to 30,000 dalton range in response to Con A, and almost all of these proteins are induction specific. Although some differences were seen among all cell lines, Tα1 and Tα2 cell lines appeared to constitute two distinct and recognizable groups (Fig. 7). In particular, the discrete band at 16,000 daltons appeared to be characteristic of Tα2 clones. A group of bands in the 17,000 to 18,000 dalton region was characteristic of Tα1 clones. Immunoprecipitation with an antisera specific for interferon-γ demonstrated that these bands were due to interferon, and that these components were absent in Tα2 samples (Fig. 8). The specificity of this antisera has been demonstrated.4

### Discussion

In our panel of antigen-specific Tα cells, we have been able to group the clones into two clearcut groups according to a variety of criteria. These include lymphokine bioactivities, helper function, and biosynthetic labeling patterns. T cell clones could be assigned to one or the other of these subclasses by using any of these three criteria, and the other properties were consistent with the assignment. The concordance of these different assays for T cell diversity, and the derivation of examples of both types of Tα cells from four different strains of mouse, suggest that these groups of Tα cells represent real subdivisions of the Tα cell class that may have important relevance in vivo.

Although the two groups of Tα cells showed very different biological activities, they shared several properties that are typical of helper cells generally. All were positive for the L3T4 surface antigen, and all lacked the Lyt-2 antigen. Examples of both types of Tα cells were restricted by or specific for the I region of the MHC, and examples of both types provide B cell help (results not shown). The lack of distinctive surface markers may have hindered past efforts to identify the two types of helper cell.

The bioactivities detected in activated Tα1 supernatants were consistent with the synthesis of IL 2, IL 3, and interferon-γ. The presence of these lymphokines in one T cell clone was also demonstrated by recombinant cloning of IL 2 (14) and Northern blot RNA analysis of IL 3 and interferon-γ (9). All three lymphokines have also been demonstrated by biosynthetic labeling and immunoprecipitation in L82-1 supernatants.4 In contrast, Tα2 clones did not synthesize detectable amounts of IL 2 or interferon-γ assayed by bioactivity, immunoprecipitation, or sequence-specific probing of a Tα2 cDNA library (K. Araki, personal communication). Tα2 clones also produced several less defined lymphokine activities that were not detected in Tα1 supernatants. These activities include IgG1- and IgE-enhancing activities and la-inducing activity, all of which may be mediated by BSF1 (18, 20–22), a T cell activity that was distinct from IL2, and a MCGF activity distinct from IL3. These latter two activities have not yet been biochemically resolved from one another, and all T lines tested make both activities or neither activity. We are currently attempting to identify the molecule(s) responsible for these activities. We also have preliminary evidence that both Tα1 and Tα2 cell
Figure 6. Bioassay of supernatants from antigen-stimulated T cell clones. Three Tα1 and two Tα2 cell lines were stimulated with antigen in the presence of antigen-presenting cells and the resulting supernatants were assayed on HT2, NSF60, and MM3 cell lines. The MM3 assay medium was supplemented with 2% Con A-induced L92-1 supernatant. All starting dilutions were 1/4. L92-1 (-- -- --), MD13-10 (-- -- --), M264-37 (-- -- --), H39-34 (-- -- --), M264-30 (-- -- --).

lines can synthesize GM-CSF, as a result of data from bioactivity assays and screening of recombinant cDNA libraries (unpublished results).  

The supernatants of the T cell clones used in these studies were rich sources of lymphokine activities. Although we used clonal assay lines to detect several of the biological activities, these assay lines did not always detect only one lymphokine. In some cases, more than one activity was detected by one cell line, and certain activities interacted with one another, either positively or negatively. Some examples include the inhibition by interferon-γ of IL-3-induced proliferation of NFS60 cells and of the IgE and IgG1, enhancing activities on LPS-stimulated B cells, the synergistic effect of IL-3 and MCGF2 on the growth of MM3 mast cells, and the response of HT2 cells to both IL-2 and a second factor that gave a signal in the HT2 assay. For the conclusions described above, we have relied mainly on “dominant”

### Table II

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<td>Tα2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1.Ly-1/9</td>
<td>57*</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>HD2-9</td>
<td>56</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>H39-34</td>
<td>13</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>H39-56</td>
<td>32</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>H39-131</td>
<td>73</td>
<td>9.1</td>
<td></td>
</tr>
</tbody>
</table>

*Con A-induced T cell supernatants were tested for their ability to enhance IgE and IgG1 production by LPS-stimulated, T-depleted spleen cells. The stimulation index is the fold level obtained with the addition of T cell supernatant, divided by the Ig level produced in control cultures stimulated only by LPS.

* IgE levels in cultures stimulated with LPS alone or LPS plus Tα1 supernatants were below the threshold of detection in the assay (1.25 mg/ml).

* For the purpose of calculation, the IgE levels in cultures stimulated by LPS alone were assumed to be 1.25 mg/ml; thus, the IgE stimulation indices represent minimal estimates.

### Table III

<table>
<thead>
<tr>
<th>T Cell Clone</th>
<th>Units of La Inducing Factor/ml</th>
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<tbody>
<tr>
<td>Tα1</td>
<td></td>
</tr>
<tr>
<td>L92-1</td>
<td>&lt;20</td>
</tr>
<tr>
<td>MD13-5</td>
<td>&gt;20</td>
</tr>
<tr>
<td>MD13-10</td>
<td>&lt;20</td>
</tr>
<tr>
<td>M264-15</td>
<td>&lt;20</td>
</tr>
<tr>
<td>M264-20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>M264-37</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Tα2</td>
<td></td>
</tr>
<tr>
<td>H39-72</td>
<td>2,000</td>
</tr>
<tr>
<td>H39-34</td>
<td>&gt;25,000</td>
</tr>
<tr>
<td>M264-35</td>
<td>200</td>
</tr>
<tr>
<td>C1.Ly-1/9</td>
<td>2,500</td>
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</tbody>
</table>

*Con A-induced T cell supernatants were tested for the induction of la antigens on B cells. One unit/ml induced 50% of maximum la induction.

### Table IV

<table>
<thead>
<tr>
<th>T Cell Clone</th>
<th>Ly-1</th>
<th>L3T4</th>
<th>Ly-2</th>
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<tbody>
<tr>
<td>Tα1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L92-1</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MD13-5</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GK15-1</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>GK15-2</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>M264-15</td>
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<td>+</td>
</tr>
<tr>
<td>M264-20</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M264-37</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tα2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M92-1</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C1.Ly-1/9</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>M264-9</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
TWO TYPES OF CLONED HELPER T CELL

TABLE V

| MHC restriction or specificity of Tα1 and Tα2 clones |
|-----------------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|
| K       | d    | b    | k     | b     | k     | k     | k     | k     | k     |
| D       | ddd  | bbd  | kkkk  | kkkk  | kkkk  | kkkk  | bbbk  | bbbk  | bbd  |

MD13-5.1  2999 a  — — — — — — ND ND | ND |
MD13-10  3133 — — — — — — ND ND | ND |
LB2-1 ND a  — 15 ND ND 4 12549 — — | ND |
LB19-1  13 2669 — — — 12 5357 — — | ND |
GK15-2  68 56 1846 1367 1411 2265 51 1259 — | ND |
MD64  95 11 5413 2881 1892 2653 ND 1336 155 27 | |
HDZ-9  64 2105 — — — — ND 11 2387 — | |
MDK3  100 35 8276 5553 3878 3991 ND 23 4737 60 | |
MDK5  26 — 741 773 1493 1443 ND 20 — 22 | |
H39-34  38 — 4229 2112 2009 2170 ND 3075 29 15 | |
H39-72  20 — 183 63 123 74 ND 134 14 — | |

a The alleles for the Aα, Aγ, Eα, and Eγ genes are shown.

ND Less than 10 U/ml.
the data of Kim et al. (6) when using T cell clones suggest
different phenotypes assessed by the pattern of B
cell help, and therefore the two major subdivisions of T
cells that have described according to lymphokine pro-
duction may be additionally subdivided when more data
are available. We have obtained data on several T1 cells
clones that suggest that these may differ in B cell helper
function (manuscript in preparation), and the patterns of
biosynthetically labeled secreted proteins suggest ad-
ditional differences between the T cell clones. Although
similarities could be seen between all T1 cells and
between all T2 cells, there were individual differences
between the clones, especially in the T2 group. The
presence or absence of some of the proteins in particular
T cell clones suggest that additional bioassays may reveal
functional heterogeneity within the T1 and T2 groups.
This is also suggested by the two-dimensional electropho-
resis results of Kettman and Lefkovits (23), who argue
for considerable T cell heterogeneity.

There appears to be some preference for the type of T
1

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T cell clone isolated in different experiments. We have
isolated CRBC-specific T cell clones from three different
mouse strains, and in over 200 antigen-specific clones
we have not found any T2 examples. In contrast, FGG-
specific clones in one experiment were all T2. Both T1 and
T2 clones were found among allotypic T1 cells specific
for the I region of the MHC. This apparent asso-
ciation of certain antigen specificities with one or another
type of T cell may represent genuine differences in the
induction of T cells in a manner analogous to the induc-
tion of different antibody isotypes by different antigens.
In fact, these two phenomena may be related, because
the T1 and T2 cells may have differential effects on
the Ig isotype produced in vitro. T2 cells produce activities
that strongly enhance IgG and IgG1 production rela-
tive to other isotypes in a polyclonal activation system,
whereas T1 cells produce interferon-γ, which strongly
inhibits the enhancing activities in T2 supernatants.2 If
the two types of T cell clones produce similar activities during
an in vivo immune response, the following hypothesis
can be advanced. An antigen, such as FGG adsorbed
to alum, that induces a substantial IgE response may do so
by preferentially inducing T1 cells, whereas CRBC,
which induce large IgM but very small IgE responses,
may do so by preferentially activating T2 cells that help
IgM but suppress IgE production. We are also investi-
gating the association of the type of T1 cell in relation to the
antigen and the adjuvant used for immunization.

The derivation of two distinct types of T1 cell clones
raises the question of the total diversity of T cell pheno-
types. The procedures used to generate our T cell clones
may be highly selective for particular types, and it is
quite possible that other T cell types exist in vivo. This
is underscored by the uniform phenotype of our anti-CRBC
T cell lines, which were all T1. Only when we examined
T cell clones specific for FGG or alloantigen did we
discover antigen-specific T cells of the second phenotype.
The study of additional antigens or alterations in culture
conditions may reveal additional types of T1 cell.

The two types of helper cells described in this report
differ in several respects, and yet they have not been
extensively described before at the clonal level. Several
features of these cells may have contributed to reducing
the number of T2 clones isolated. Because T2 cells are
dependent on IL-2 for growth, and yet do not produce IL-
2, these cells must be grown on a constant supply of
exogenous IL-2. In addition, we have found that the T2
clones do not grow as rapidly as T1 clones, and they
may have extra requirements for growth or a limited
life span. We have been much more successful in growing
large numbers of T1 clones than T2 clones.

Several questions about the two types of T1 cells now
need to be answered. Because both types bear the same
cell surface markers as the delayed-type hypersensitivity
(DTH) effector cells, is one or both types identical to the
DTH cell? What is the relative contribution of each type
of T cell to normal in vivo responses? Do the differences
between T1 and T2 cells represent separate lineages of
T1 cells, or do the two types represent different stages of
the same lineage? Is the apparent selectivity of the anti-
gen or adjuvant for T1 or 2 a real phenomenon, and
what determines this selectivity? How is IL-3 regulated
 coordinately with IL-2 and interferon-γ in T1 cells and
yet noncoordinately in T2 cells? The T cell clones de-
scribed in this report should be useful in answering
these and many other questions.

Acknowledgments. We thank J. Christiansen, J.
Carty, D. Cher, and J. Tomaszolo for valuable technical
assistance, G. Zurawski and K. Araki for generous gifts of
recombinant IL-2 and cDNA clones respectively, and J.
Zahner and G. Barget for assistance in preparing the
manuscript.

Note Added in Proof: We have recently obtained evi-
dence from serological data and recombinant DNA cloning
of BSF-19 that the activities described as MCGF2 and
TCGFG2 are mediated by BSF-1.

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