T-CELL REGULATION OF HUMAN PERIPHERAL BLOOD B-CELL RESPONSIVENESS

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The interactions necessary for the generation of mature plasma cells from precursor B cells are not entirely understood, but evidence suggests that antigens, T cells, and macrophages are involved (1–7). It has become increasingly clear that T lymphocytes exert critical regulatory control on the immune responses of B lymphocytes as well as other T lymphocytes (8). The signals originating from T cells and their products have been most extensively studied and the following points have emerged: (a) the cell interactions between T and B cells are genetically restricted by genes coded for, or closely linked to the major histocompatibility complex (MHC) of the species (9, 10) and (b) the influence of T cells may be of a positive (helper) (11) or negative (suppressor) (12) type. It is thought that T-cell regulation is most critical during both the induction of a primary, and in the elicitation of a secondary antibody response. At present, little evidence exists for a role of T cells in the regulation of synthesis of antibodies by more differentiated B cells or plasma cells.

In the present studies, we have investigated the influence of T cells on the spontaneous synthesis and secretion of Ig by human peripheral blood B cells. Although the specificity of the immunoglobulins are unknown, B-cell production of Ig can be readily detected by a reverse hemolytic plaque technique which detects Ig secretion through the use of anti-Ig-coated erythrocytes followed by development with complement. The experiments described below show that: (a) peripheral blood lymphocytes contain cells which actively synthesize and secrete Ig; (b) highly purified B cells alone, in contrast, are less capable of secreting Ig than unfractionated lymphocytes; (c) maximal secretion of Ig by B cells can be reconstituted by the addition of T cells; and (d) these T-B interactions appear to be influenced by compatibility at the MHC.

Materials and Methods

Isolation of Purified T and B Cells from Human Peripheral Blood. Mononuclear cells were isolated from the peripheral blood of normal volunteers by Ficoll-Hypaque density gradient centrifugation. These unfractionated cells were separated into surface Ig+ B cells and surface Ig− T-cell populations by methods previously described (13). Briefly, cells were passed over Sephadex G-200 columns which had been coupled with anti-human F(ab)2 antibodies. Cells which were surface Ig+ passed through the column, whereas surface Ig− cells were retained and subsequently...
eluted with human gamma globulin. Both the Ig^+ and Ig^- populations were analyzed by direct rosetting with human erythrocytes coated with anti-light-chain antibody (14). Ig^+ cells were shown to form at least 95% rosettes, whereas Ig^- populations had <1% rosetting cells. In previous experiments (13) it has been shown that <2% of cells retained on anti-F(ab)_2 columns form E rosettes, whereas >80% of the nonretained cells will form rosettes with sheep erythrocytes. In addition, <2% of Ig^- cells stain positively for intracellular immunoglobulin.

**Mixing of Cells.** Both Ig^- and Ig^+ cells were thoroughly washed in RPMI 1640 media (Grand Island Biological Co., Grand Island, N. Y.), and for most experiments, mixed at ratios of 70% T cells and 30% B cells at a final concentration of 1 x 10^6 cells/ml. In studies quantitating the number of autologous T cells required to trigger B-cell Ig synthesis and secretion, varying percentages of T cells were added to the B-cell population and the final mixture contained 1 x 10^6 cells/ml. In these studies it was assumed that all surface Ig^- cells in the B-cell population (generally 3%) were T cells.

**The Reverse Hemolytic Plaque Assay.** Ig-secreting cells were detected by a reverse hemolytic plaque assay which has been previously described in detail (15, 16). Briefly, 50 µl of an 11% suspension of sheep erythrocytes, which were previously coated with rabbit anti-human Ig and 50 µl of lymphocytes, was pipetted into 10 x 75-mm glass test tubes containing 0.9 ml of an 0.8% solution of Sea-Plaque agarose (Marine Colloids, Rockland, Maine) in Hanks' Balanced Salt Solution (HBSS, Grand Island Biological Co.). The tubes were mixed and layered over 5 ml of gelled 1.0% Sea Kern agarose (Marine Colloid) in HBSS in a 60-mm Petri dish. After the top layer had gelled, the Petri dishes were incubated for 1 h at 37°C in a humid atmosphere containing 5% CO₂. 1 ml of rabbit anti-human Ig antisera diluted in HBSS was pipetted onto the Petri dishes, incubated for 1 h at 37°C, and 1 ml of guinea pig complement (Grand Island Biological Co.) diluted 1:10 was added. Incubation was continued for an additional hour and the plaques were counted. The plates could be stored at 4°C for 24 h if necessary.

**HLA Typing.** Families used in these studies were typed by Dr. Edmund Yunis at the Sidney Farber Cancer Institute, with 144 well-characterized anti-HLA sera which included reactivity against HLA-A, HLA-B, and HLA-C antigens using the National Institutes of Health monocyte-toxicity technique (17). HLA typing was obtained after plaque formation results had been ascertained.

**Results**

**Spontaneous Ig Synthesis by Normal Peripheral Blood Lymphocytes.** The spontaneous plaque-forming response of unfractionated, Ig^- and Ig^+ cells from five different individuals is shown in Table I. In the unfractionated cell populations, the number of plaque-forming cells (PFC) per 10^6 cells ranged from 7,900 to 12,800, averaging 10,664 ± 1,851, whereas purified Ig^- cells showed virtually no plaque-forming activity. Unexpectedly, column-purified Ig^+ cells also showed a striking decrease in the number of PFC.

Since unfractionated lymphocytes contained greater numbers of PFC than either purified Ig^+ or Ig^- cells alone, we investigated the PFC response of reconstituted populations. Lymphocytes were obtained from three individuals and purified T cells were added to B cells in graded amounts so that the final percentage of added T cells varied from 4 to 18% (Fig. 1). Increases in plaque formation were seen when as few as 6-8% T cells were added. In all individuals tested, the plaque-forming response of reconstituted samples was similar to that of unfractionated cells providing 10-15% Ig^- cells had been added back to purified Ig^+ cells (Fig. 1). These studies suggest that Ig^- cells could enhance the Ig-secreting capacity of Ig^+ B cells. We would emphasize that the lymphocytes used in these experiments were not triggered with mitogens or cultured for a time greater than that necessary to complete the assay (3 h).

**Cycloheximide Treatment of Purified Ig^- and Ig^+ Cells.** To investigate the requirement for active protein synthesis, cycloheximide (50 µg/ml) or media
Table I
Plaque Formation with Unfractionated T- and B-Lymphocyte Populations

| Individual | Unfractionated | T*  | B*  |
|------------|----------------|-----|-----|
| 1          | 10,200         | 10  | 450 |
| 2          | 11,800         | 0   | 320 |
| 3          | 12,800         | 3   | 3,800|
| 4          | 7,900          | 0   | 400 |
| 5          | 10,620         | 0   | 680 |

Mean ± SE 10,664 ± 1,851 1,130 ± 1,498

* Plaques/10⁶ cells.

![Graph]

Fig. 1. Quantitation of T-cell help for maximal secretion of immunoglobulin. Purified Ig⁻ and Ig⁺ cells from each of three individuals were mixed together in graded amounts such that the percentage of autologous Ig⁻ cells varied between 11 and 18%. Each mixture was then assayed for the number of PFC per million cells plated. Unfractionated Ficoll-purified lymphocytes were used to determine control levels of immunoglobulin-secreting cells. A, O, O, unfractionated lymphocytes alone were added to purified Ig⁻ or Ig⁺ cells for 1 h at 37°C. The cells were washed and tested alone or in mixtures, and the plaque-forming response was determined and compared to the response of unfractionated lymphocytes. As is shown in Table II, treatment of highly purified Ig⁺ cells with cyclohexamide virtually abrogated the PFC response. In contrast, pretreatment of purified Ig⁻ cells with cycloheximide had no effect on the secretion of Ig by untreated B cells in reconstituted populations (Table II). These studies suggested that active protein synthesis was necessary only in the B-cell population.

Evidence for Genetic Restriction of T-Cell Help for Maximal Secretion of Immunoglobulin. To determine whether or not allogeneic T-cell populations could reconstitute B-cell plaque formation, mixtures of Ig⁻ and Ig⁺ cells from three individuals were tested and compared to autologous mixtures (Figs. 2 A and B). T cells from individual X (Tx) were mixed with autologous B cells (Bx) and 7,800 PFC/10⁶ were obtained at a ratio of 70% Tx to 30% Bx cells (Fig. 2A). In contrast, when Tx cells were mixed with B cells from unrelated individuals (By or Bz cells) a marked reduction in plaque formation was seen (Fig. 2A). Similar reductions were observed when Ty cells and Bx or Bz cells were mixed, whereas maximal levels were again obtained with autologous combinations of cells (Fig. 2B).
HUMAN T- AND B-CELL COOPERATION

Table II

The Effect of Cycloheximide Treatment of Purified T and B Cells on Plaque Formation

| Lymphocyte populations                                      | Plaques/10^5 cells |
|-------------------------------------------------------------|--------------------|
| T Cells (cycloheximide) + B cells (cycloheximide)           | 940                |
| T Cells (cycloheximide) + B cells                           | 7,800              |
| T Cells + B cells (cycloheximide)                           | 801                |
| T Cells + B cells                                           | 9,440              |
| B Cells                                                     | 3,080              |
| B Cells (cycloheximide)                                     | 120                |
| Lymphocytes (unfractionated)                                | 9,480              |

Fig. 2. Cooperation of autologous and allogeneic T cells in the secretion of immunoglobulin by B cells. Column-purified B cells from three individuals, X, Y, and Z, were tested for reconstitution of plaque-forming abilities with purified T cells from individual X (A) and individual Y (B). Ratios of T and B cells were constructed such that the T cells varied from 3 to 99% and B cells from 97 to 1%.

These studies suggested that autologous T cells cooperated best in the reconstitution of plaque formation. To determine whether or not optimal cooperation was influenced by the MHC, we examined T- and B-cell interactions in two families whose haplotypes were determined by HLA typing of the A, B, and C loci (Figs. 3 and 4). Each of the reconstituted samples contained ratios of 70% Ig^- and 30% Ig^+ cells, and the number of PFC per million lymphocytes was represented as a percentage of the plaque-forming response found with autologous Ig^- and Ig^+ cells.

In family A, individuals 3 and 5 shared identical haplotypes as did individuals 6 and 7 (Fig. 3). Similarly, individuals 1 and 4 in family B and individuals 2 and 5 shared identical haplotypes (Fig. 4). The plaque-forming response of reconstituted populations within these families is shown in Tables III and IV. It can be seen that in all cases, the plaque-forming response of autologous mixtures of Ig^- and Ig^+ cells was comparable to that obtained by unfractionated cells. Furthermore, Ig^- and Ig^+ cells from individuals 3 and 5, and 6 and 7 in family A and 1 and 4, and 2 and 5 in family B cooperated in most cases better than individuals who did not share identical haplotypes. There are some
Fig. 3. Haplotypes of the members of family A as represented by HLA typing of the A, B, and C loci.

Fig. 4. Haplotypes of the members of family B as presented by HLA typing of the A, B, and C loci.

unexpected results seen in the studies done with these families. For example, although Ig- cells from the parents of family A cooperated well with the Ig+ cells of each of the siblings and less well with one another, similar results were not observed in family B. In addition, in both families studied, Ig- cells from one of the siblings were able to cooperate very well with Ig+ cells from several of the other members of the family (individual 3 in family A, individual 1 in family B).

Discussion

The studies reported here demonstrate that: (a) unfractionated peripheral blood lymphocytes contain variable numbers of cells spontaneously secreting Ig; (b) purified T cells lack the ability to secrete immunoglobulin and form plaques; (c) Ig secretion by B cells alone is markedly reduced; (d) T cells are required for maximal levels of Ig synthesis and secretion by B cells; (e) treatment of purified T cells by cycloheximide at concentrations known to inhibit protein synthesis does not inhibit the cooperative potential of these cells; and (f) autologous Ig- and Ig+ cells cooperate markedly better than allogeneic combinations of Ig- and Ig+ cells.

The assay used to identify PFC measures Ig secretion, is a short-term assay, and requires no exogenous stimulation. Despite this, T cells are potent regulators of plaque formation, since as few as 10–15% T cells can reconstitute maximal plaque formation by B cells. The mechanism involved in this helper effect is unknown, but the cycloheximide data suggests that newly synthesized mediator production may not be involved. Either preformed mediators already
present in T cells may affect this helper phenomenon or, alternatively, cell surface interactions may be responsible for the observed effect. Similar cell surface interactions regulated by products of the MHC have been extensively described (18, 19). On the other hand, the interactions at the cell surface could be the result of Fc receptors which have been shown to be on the surface of human T cells (20, 21). The observation that autologous T cells cooperate in most cases better than allogeneic T cells (Figs. 2A and B) from randomly selected individuals, makes the possibility of nonspecific surface interactions such as those mediated by Fc receptors less likely. Moreover, in studies done on two families we found that Ig- cells from HLA-identical siblings cooperated in a manner comparable to autologous T cells, supporting the view that genetic restrictions are involved in T- and B-cell cooperation. Still to be explained is the observation that Ig- cells from one individual in each family seem to

**HUMAN T- AND B-CELL COOPERATION**

**Table III**

Reconstitution of Plaque Formation* in Family (A) Containing Two Pairs of HLA-Identical Siblings

| B Cells (30%) |
|--------------|
| Donor | T Cells (70%) |
| 1 | 2 | 3 | 5 | 6 | 7 | 9 | 10 |
| T Cells (70%) | 100 | 98 | 79 | 63 | 51 | 52 | 52 | 37 | 100 |
| 2 | 25 | 100 | 52 | 36 | 45 | 31 | 48 | 38 | — |
| 3 | 100 | 88 | 100 | 103 | 75 | 102 | 105 | 90 | 85 |
| 5 | 43 | 27 | 109 | 100 | 61 | 52 | 48 | 59 | — |
| 6 | 34 | 30 | 70 | 27 | 100 | 92 | 69 | 44 | 95 |
| 7 | 96 | 87 | 107 | 76 | 104 | 100 | 44 | 113 | |
| Maternal | 122 | 80 | 107 | 98 | 90 | 102 | 100 | 78 | 112 |
| Paternal | 108 | 96 | 113 | 92 | 113 | 102 | 56 | 100 | 88 |

* Values are represented as the percent of plaque formation found with mixtures of autologous T and B cells.

**Table IV**

Reconstitution of Plaque Formation* in Family (B) Containing Two Pairs of HLA-Identical Siblings

| B Cells (30%) |
|--------------|
| Donor | T Cells (70%) |
| 1 | 2 | 3 | 4 | 5 | 6 |
| T Cells (70%) | 100 | 90 | 122 | 134 | 134 | 98 | 101 |
| 2 | 36 | 100 | 46 | 23 | 112 | 15 | 96 |
| 4 | 93 | 72 | 82 | 100 | 75 | 60 | — |
| 5 | 44 | 94 | 30 | 64 | 100 | 85 | 110 |
| Maternal | 74 | 69 | 100 | 87 | 104 | 34 | 94 |
| Paternal | 29 | 15 | 98 | 14 | 12 | 100 | 98 |

* Values are represented as the percent of plaque formation found with autologous T and B cells.
cooperate with all the cells from members of that family. The mechanism for these types of interactions within families is presently unclear. However, it should be noted that the D locus was not characterized in the individuals studied in these two families and this locus may have a bearing on the interactions observed between individuals who were nonidentical at the HLA-A, HLA-B, and HLA-C loci. Studies utilizing typing of the D loci and recombinant members of certain families are currently in progress to resolve this point. These studies may allow us to better define those areas of the MHC which may be involved in T- and B-cell cooperation.

The regulatory role of T cells upon B-cell function has been investigated most closely during the early stages when B-cell triggering occurs upon presentation of antigen. In contrast, the data described above indicate that T cells play a regulatory role in the latter stages of B-cell differentiation as well.

Summary

We have investigated the influence of human T cells on the synthesis and secretion of immunoglobulin by peripheral blood B cells. The plaque-forming assay used, which identified the number of B cells secreting Ig, is a short-term assay which requires no exogenous stimulation. We have shown that the B-cell population alone contains fewer secreting cells than the total lymphocyte population, and that T cells are required to achieve maximal plaque-forming cell levels. Cycloheximide treatment of cells at concentrations known to inhibit protein synthesis does not affect the cooperative potential of these cells. Additionally, this cooperation effect is markedly better among autologous mixtures of Ig- and Ig+ cells, than among mixtures obtained from randomly selected individuals.

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1772  HUMAN T- AND B-CELL COOPERATION

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