SPECIFICITY AND FUNCTION OF A HUMAN AUTOLOGOUS REACTIVE T CELL*

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Several groups have recently reported the existence of a population of human T cells capable of proliferating in response to signals from autologous, non-T, mononuclear cells (1-4). As the stimulator cells have not been purposely modified, this proliferation may represent recognition of unaltered self determinants. In this report, we have investigated the specificity and function of this autologous, reactive T cell (ARC). The data indicate that the ARC is: (a) activated by products of genes closely linked to the HLA-DR locus of the major histocompatibility complex (MHC), and (b) required for the synthesis of substantial amounts of Ig by autologous B cells. This suggests that the ARC is a helper cell capable of recognizing self MHC determinants.

Materials and Methods

Cell Populations and Culture. Whole peripheral blood mononuclear cells (PBMC), T-enriched populations (92 ± 4% T, 3 ± 1% Ig-bearing, 4 ± 2% esterase-positive cells), populations enriched for B cells and monocytes (referred to as "B cells"); 40 ± 8% Ig-bearing, 40 ± 10% esterase-positive, 10 ± 3% T), as well as T-cell populations differing in their density, were obtained as previously described (5, 6).

Reactivity to autologous and allogeneic cells was determined by culturing (37°C; in 95% air, 5% CO2) 100,000 T-enriched responder cells with 50,000 mitomycin-C-treated (mitomycin C, Sigma Chemical Co., St. Louis, Mo.; 50 µg mitomycin C/10^6 cells) B cells in RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.) containing serum autologous to the responding cell (human autologous serum [HAS]) (10% HAS, heated at 56°C for 30 min). Proliferation was assayed by determining the amount of tritiated thymidine (3H-TdR; 2 Ci/mM, New England Nuclear, Boston, Mass.) that was incorporated into DNA during the final 18 h of a 6-d culture. Results are expressed as stimulation index (SI, counts per minute of cultures containing responders and stimulators/cells per minute of responders tested alone plus counts per minute of stimulators incubated alone).

To negatively select for ARC, 3 × 10^6 T-enriched populations were cultured with 1.5 × 10^6 mitomycin-C-treated, autologous B cells in 10% HAS. At 72 and again at 96 h of culture, 5-bromo-2-deoxyuridine (BUDR, Sigma Chemical Co.) was added in a final concentration of 3.3 µg/ml. 18 h after the final addition of BUDR, all cultures were exposed to cool white light at 23°C for 2 h, and the recovered, washed cells tested for (a) their subsequent proliferative response to autologous or allogeneic stimulator cells and (b) their ability to regulate Ig

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Abbreviations used in this paper: ARC, autologous reactive cell; BSA, bovine serum albumin; BUDR, 5-bromo-2-deoxyuridine; HAS, human autologous serum; 3H-TdR, tritiated thymidine; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cells; PWM, pokeweed mitogen; SI, stimulation index.

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Ig synthesis occurring during a 7-d culture of cells with two concentrations of pokeweed mitogen (PWM, Grand Island Biological Co., 6.25 and 1.56 μg/ml) was determined utilizing a competitive-binding radioimmunoassay, as previously described (5). Results are expressed as maximal nanograms of Ig synthesized/10⁶ cells, i.e., that which resulted from exposure to the optimal concentration of PWM.

Results

As an initial step to determine the function of the ARC, T-enriched fractions were sedimented on a five-step discontinuous bovine serum albumin (BSA) gradient (10%/23%/26%/29%/33% BSA) and cells from various fractions compared for their proliferative reactivity to autologous stimulators as well as for their ability to modulate Ig synthesis by autologous B cells (Fig. 1; fractions 1 + 2, 10%/23%, 23%/26% interfaces were pooled as were fractions 4 + 5, 29%/33% interface, and pellet, in order to obtain sufficient numbers of cells). Substantial reactivity to autologous, stimulator cells (SI ≥ 4) was noted among T cells localizing to fractions 1 + 2, or 3, but not among cells localizing to fractions 4 + 5 (SI < 2). Low-density fractions containing ARC also augmented Ig production by autologous B cells (2.6- and 2.1-fold, respectively), although high-density cells lacking ARC did not (Fig. 1B). In data not shown, it could be demonstrated that these high-density cells suppressed Ig synthesis by autologous whole PBMC but did not inhibit the ARC reactivity among low-density fractions.

To more directly demonstrate that the ARC may function as a helper rather than as a suppressor cell, advantage was taken of the fact that proliferating cells can be suicided with BUDR and light (7). The utility of this as a method for selectively depleting ARC is indicated by data in Table I. T cells were cultured with autologous B cells, and one-half of the cultures received BUDR. The washed, recovered cells were then tested at equivalent concentrations of viable cells for their subsequent
T cells from individual A were cultured with mitomycin-C-treated (m) autologous B cells in the absence (−) or presence (+) of BUDR. All cultures were exposed to light. The washed, recovered cells were then resuspended to comparable concentrations of viable cells and tested for their subsequent proliferative response to either autologous B cells or B cells from two other individuals (B, C). The HLA-A, B, and DR phenotypes of the cells are indicated. Reactivity was measured by the incorporation of ³H-TdR into DNA and is expressed as the SI. The results are representative of three experiments. Tissue typing was performed utilizing antisera directed against eight different DR determinants (DR-1–DR-8). (−) refers to a lack of reactivity with any of these antisera.

Reactivity to stimulator cells bearing known HLA-A, B, or DR phenotypes. Populations which had been negatively selected for proliferating ARC (A + Am + BUDR, Table I) demonstrated a 67% decrease in their subsequent reactivity to autologous stimulators (individual A, Table I) but no decrease in their response to stimulators sharing a single HLA-A and B, but not a definable DR, determinant (individual B, Table I). In contrast, the subsequent reactivity of the ARC-depleted population to stimulators disparate with regard to HLA-A and B determinants, but which did share one DR determinant (individual C, Table I), was decreased by 8%. These data indicate that suicide with BUDR and light does provide a method for selective deletion of ARC. Furthermore, they suggest that the ARC is responsive to signals coded for by genes more closely linked to the HLA-DR, than to the HLA-A, or B, locus.

Populations containing or negatively selected for ARC were next compared, in three ratios, for their modulating effect on the PWM-induced Ig synthesis by either autologous B cells or whole PBMC (Fig. 2). ARC-containing cells (T + B − BUDR) substantially augmented Ig production by autologous B cells at each ratio tested (11-, 24-, and 8.4-fold increases in Ig synthesis at 9/1, 3/1, and 1/1 ratio of T/B, respectively, Fig. 2A). In contrast, populations depleted of ARC demonstrated diminished helper activity.

Three points concerning the relative loss of help among the ARC-depleted population deserve emphasis. First, it did not simply reflect inhibition of Ig synthesis caused by "carry over" of BUDR. T cells that went through the negative selection procedure (T + B + BUDR) but that were not exposed to light did not inhibit Ig synthesis (13,000 ng of Ig/10⁶ B cells) compared to controls (T + B − BUDR, 10,500 ng of Ig/10⁶ B cells) when each regulatory population was tested at a ratio of 3/1 with autologous B cells, data not shown. Second, the greater Ig synthesis noted by mixtures of B cells and control T cells (T + B − BUDR) was not related to Ig production by the "carried over," stimulator B cells. Maximal PWM-induced Ig synthesis by the control population tested alone was meager (13% of that noted when they were mixed at a 3/1 ratio with fresh B cells; mean of five experiments). Third, the apparent absence of help among the ARC-depleted population did not represent
Fig. 2. Regulatory effect of ARC on Ig synthesis. Control populations (○) and populations negatively selected for proliferating ARC (©) were mixed in the indicated final ratios with autologous B cells (A) or whole PBMC (B). 100,000 cells from each mixture were then assayed for PWM-induced Ig synthesis. Results are presented as maximal nanograms of Ig synthesized per 10⁶ B cells (A) or per 10⁶ PBMC (B) and are representative of three experiments. (-) on the ordinate indicates the mean amount of Ig synthesized by B cells or PBMC alone.

Discussion

Several reports have now confirmed the existence of a population of human peripheral blood T cells capable of proliferating in response to signals from autologous B cells and monocytes (1-4). Although there has been no direct data, several observations have indirectly indicated that this ARC functions to regulate immunologic reactivity. On the one hand, the findings that ARC reactivity is decreased in patients with systemic lupus erythematosus (3, 4, 8), a disorder presumed to be accompanied by a deficiency of immunosuppressive influences; and is increased among umbilical cord blood lymphocytes (9), a mononuclear cell population manifesting increased T-dependent immunosuppression (10); suggest that it functions as a suppressor cell. On the other hand, there exist observations to suggest that the ARC may augment immune reactivity. Vande Stouwe et al. (11) demonstrated that activation of the ARC could help in the generation of alloreactive, cytotoxic T cells. Moreover, two laboratories have reported diminished ARC reactivity in patients with chronic lymphocytic leukemia (8, 12), a disorder in which there may be a deficiency of helper T cells (13). The studies presented in this report substantiate the concept that the ARC is involved in immunoregulation and support its putative role as a helper cell.

Crucial to the conclusion that the ARC provides help for Ig synthesis are the negative selection experiments outlined in Fig. 2. As net regulation presumably reflects a balance between help and suppression, it might be expected that this helper-cell depleted population should suppress Ig synthesis. This did not occur (Fig. 2 B) and
may be related to either the finding that negatively selected populations manifest a small degree of residual, autologous reactivity (Table I), or to the possibility that the ARC represents only one of two cooperating T cells required to provide B-cell help (14–17).

Previously, we reported that the ARC could be activated by autologous as well as allogeneic cells (18). This contrasts with data presented here (Table I). However, the previously published data utilized fetal calf serum in the culture medium, while the present studies utilized serum autologous to the responder cell. In data derived from experiments not presented here, it is clear that ARC specificity is crucially dependent on the source of the serum used in the culture medium and that at least a portion of the reactivity generated in fetal calf serum is dependent on interactions between serum constituents and cell-surface determinants which are not HLA restricted. Although somewhat incomplete with regard to DR typing, the data presented in Table I suggest that the activation of the ARC is dependent on products of genes more closely linked to the HLA-DR than to the HLA-A, or B, locus. If this is indeed the case, one might argue that the subsequent response of ARC-depleted populations to stimulators sharing one DR determinant would theoretically be reduced by 50%, rather than by 8%, as demonstrated in Table I. However, one could also argue that depletion of T cells responsive to a given DR determinant could result in a relative enrichment for T cells responsive to products of other DR loci. Indeed, after depletion of the ARC, the subsequent reactivity of the remaining cells to stimulators not sharing a definable DR determinant was increased 1.1-fold (reactivity to individual B, Table I). The finding that the subsequent reactivity of ARC-negative populations to stimulator cells sharing a DR determinant was decreased and not increased, coupled with our findings that the data presented in Table I is reproducible, strongly suggest that signals activating the ARC are more closely linked to the HLA-DR than to the HLA-A and B loci. Whether the specificity of the ARC reactivity depicted in HAS truly represents a response to unaltered, self-MHC-determinants, to self-MHC-determinants seen in conjunction with another, unidentified antigen, or to MHC determinants modified either in vivo or in vitro, remains to be determined.

Summary

Normal human peripheral blood contains a population of T cells (autologous reactive cells [ARC]) capable of proliferating in response to signals from autologous B cells and monocytes. Selective suicide of proliferating ARC with 5-bromo-2-deoxyuridine and light demonstrated that this ARC was responsive to signals coded for by genes more closely linked to the HLA-DR, than to the HLA-A, or HLA-B, loci. Density-gradient fractionation of T cells indicated that populations enriched in ARC reactivity were also enriched for helper influences required for Ig synthesis by autologous B cells. In contrast, populations negatively selected for proliferating ARC were deficient in helper activity. These studies indicate that the ARC is responsive, at least in part, to products of genes closely linked to the HLA-DR locus and can function as a helper cell.

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