Brief Definitive Report

SHEEP RED BLOOD CELL-SPECIFIC HELPER ACTIVITY IN RAT THORACIC DUCT LYMPHOCYTE POPULATIONS POSITIVELY SELECTED FOR REACTIVITY TO SPECIFIC STRONG HISTOCOMPATIBILITY ALLOANTIGENS*

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Studies with several experimental models have demonstrated the surprisingly high frequency of cells in the recirculating T-lymphocyte pool specifically reactive to one or another of the strong alloantigens of the major histocompatibility complex (MHC). These include: (*a*) 3–6% of parental strain lymphocytes stimulated to proliferate in the mixed lymphocyte interaction (MLI) and which induce local graft-versus-host (GVH) reactions (1, 2), (*b*) selective retention of up to 12% of parental lymphocytes in the tissues of F_1 hybrids during the course of a systemic GVH reaction (3), and (*c*) 6% of parental T cells bearing surface receptors which share idiotypic determinants with IgG fractions of alloantibody specific for a chosen MHC haplotype (4).

Considering the number of different MHC haplotypes that has already been identified in mice and rats (see 5), it would appear that all or nearly all of the T cells comprising the recirculating lymphocyte pool possess specificity for one or another of the strong alloantigens of the species. Such a premise raises the important question of the identity of the cell and the nature of the receptor responsible for T-lymphocyte-mediated immune responses to the universe of nonhistocompatibility antigens.

One approach to this problem is to prepare positively selected populations of lymphocytes, as recently described by Howard and Wilson (6 and footnote 1) and Binz and Wigzell (7), in which reactivity to a chosen MHC haplotype has been specifically enriched. It is then pertinent to determine the immune potential of T cells in such alloantigen-selected lymphocyte populations for immune reactivity to nonhistocompatibility antigens. In this communication, we present the results of preliminary studies which indicate that rat thoracic duct lymphocyte (TDL) populations which have been positively selected and have specifically enriched MLI and GVH reactivity to a selected MHC haplotype nevertheless possess quantitatively normal helper activity for the generation of primary sheep red blood cell (SRBC)-specific plaque-forming cells (PFC).

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Materials and Methods

Animals. Rats used in these studies were of the August 28807 (AUG; Ag-B5), Brown Norway (BN; Ag-B3), Wistar Furth (WF; Ag-B2), and the DA (Ag-B4) strains and their F_1 hybrids. T-cell deprived (B) rats were used as intermediate hosts for the temporary storage of selected lymphocyte populations described below; the preparation of B rats and assessments of their extent of T-cell deprivation have been described before (8 and footnote 1). These animals were reirradiated (300 R) before transfer of MLI-activated TDL populations to reduce the number of recirculating B cells in the lymph.

Positive Selection. The preparation of positively selected TDL populations in bulk MLI cultures, their storage in and recovery from syngeneic B rats, assessments of their enriched reactivity to chosen MHC haplotypes in analytical MLI and GVH assays, and procedures for determining the proportion of T and B lymphocytes have all been described elsewhere (6, 9, 10 and footnote 1).

In the present studies, AUG strain TDL (250×10^6) were stimulated with AUG/WF spleen cells (150×10^6) in bulk MLI cultures, the surviving cells were parked in reirradiated syngeneic B rats (80×10^6 into each of two B rats) for a period of 2 wk and then recovered by overnight thoracic duct drainage. For control purposes, similar numbers of unselected, normal AUG TDL were stored and recovered from an additional group of two B rats. Each population was then subdivided into two portions. In one of these the proportion of T and B cells was determined with cytotoxic antisera specific for rat peripheral T and B cells (9, 10) and guinea pig complement, and the degree of positive selection achieved was tested in the analytical MLI and in the local popliteal lymph node weight GVH assay (6 and footnote 1). The remaining portion of each TDL population was tested for helper activity, described below, after treatment with anti-B serum and guinea pig complement to remove contaminating B cells. The yield of T cells recovered from the B-rat recipients was generally 50% of the number of viable MLI-activated TDL injected 2 wk earlier.

SRBC-Stimulated T- and B-Cell Cultures. The culture system employed to generate SRBC-specific PFC by stimulation of recombined populations of T and B cells from rat TDL and the procedures for enumerating PFC have been described elsewhere (11–13). T cells were prepared from TDL populations of normal AUG donors or from AUG B rats reconstituted with normal syngeneic TDL or with MLI-activated TDL. B cells were obtained from the TDL of AUG strain B-rat donors.

Previous studies have demonstrated the dose-dependent helper activity of T cells from normal rat TDL in the generation of SRBC PFC from a constant number of B cells in culture. Expressed on log-log plots, PFC responses show a positive linear regression in the range of 10^4–10^5 syngeneic T cells.

Results

Preliminary Considerations: Positive Selection of T Cells with Reactivity to a Specific MHC Haplotype. Fig. 1 shows that the AUG anti-WF MLI-activated TDL stimulated after recovery from B rats responded in analytical MLI cultures to a much greater extent to alloantigens of the WF haplotype than to third party (BN) alloantigens. Furthermore, in analytical GVH reactions the relative potency of the positively selected populations was approximately 10, a figure very similar to that obtained in previous studies (6 and footnote 1). Whereas 1.0–1.5×10^6 unselected AUG T cells were required to give a 20 mg lymph node, comparable responses were achieved with only 0.15×10^6 T cells in the AUG anti-WF population.

SRBC Helper Activity in Normal and Alloantigen-Selected T Populations. The straight line in Fig. 2 A, B represents the regression line for helper responses of normal T cells (nonparked) obtained from eight independent experiments conducted at different times (11). Helper activity of the AUG anti-WF MLI T cells (Fig. 2 A), described above, and of an additional population of AUG anti-DA T cells, tested at a different time (Fig. 2 B), were quantitatively similar to the helper responses of normal, unselected AUG T cells stored in B rats.

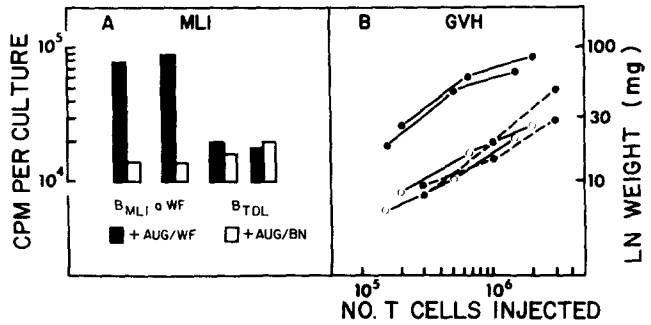

FIG. 1. (A) Proliferative responses of parked populations of AUG anti-WF MLI TDL and normal AUG TDL-stimulated with specific (AUG/WF, ■) or with third party (AUG/BN, □) splenic cells. The cultures were terminated on day 5 after exposure to [^{3}H]TDR. (B) GVH reactivity of parked populations of AUG anti-WF MLI TDL (●) and normal AUG TDL (○) tested in specific (AUG/WF, ——) and third party (AUG/BN, - - -) F_1 recipients. The number of T cells injected into each foot pad is indicated on the X axis. Each point represents the mean weight of three lymph nodes; the standard errors around each were less than 20% of mean values and are not indicated for the sake of clarity.

These results indicate that positively selected AUG TDL populations possess helper activity for SRBC responses which is comparable in magnitude to that present in both parked and nonparked populations of normal T cells. In this experiment, no PFC responses were obtained in the absence of SRBC in the cultures nor in separate cultures of the various T and B populations in the presence of SRBC.

Discussion

The specificity of immune responses involving activation of relevant, antigen-specific lymphocytes has generally been accounted for by a minimal model based on one cell, one receptor, and reactivity to one antigenic determinant. Implicit in this simplistic model is the notion that cells reactive to one antigenic determinant are not triggered by another except when it is sufficiently cross-reactive with the first to bind to the receptor and cause activation. In this context, the high frequency of T cells bearing receptors for (4) and reactive to (1–3) strong alloantigens of the MHC are findings not easily reconciled with the fine specificity and extensive diversity of T-cell responses to nonhistocompatibility antigens.

The present experiments demonstrate that positively selected T-cell populations, having MLI and GVH reactivity for a chosen MHC haplotype which is specifically enriched 10-fold, also possess quantitatively normal helper activity for collaboration with B cells in primary responses to SRBC antigens. It can be concluded from this finding that T-helper cells for SRBC responses, and by implication T cells reactive to other nonhistocompatibility antigens, are included in the T-cell population reactive to strong histocompatibility alloantigens, and that they do not belong to different T-cell subsets. This conclusion follows from our interpretation of the cellular basis of positive selection of specific alloantigen-reactive cells; namely, that enriched reactivity to a chosen MHC haplotype achieved in preparative MLI cultures depends on both the

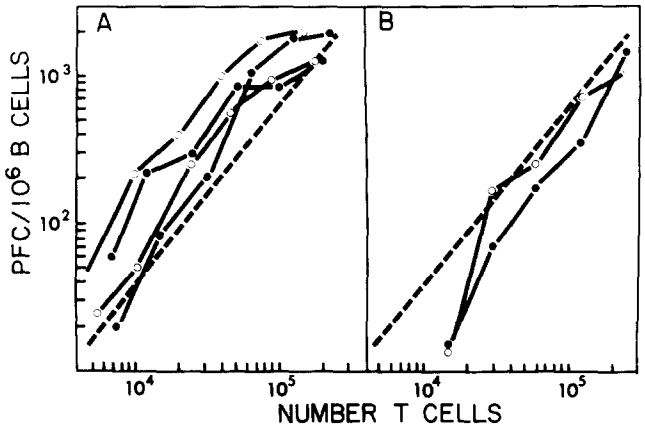

FIG. 2. SRBC-PFC responses by rat B cells cultured with titrated numbers of T cells from parked populations of AUG anti-WF MLI TDL (●, Fig. 2 A), AUG anti-DA MLI TDL (●, Fig. 2 B), and normal (unselected) AUG TDL (○). The straight line is a regression curve drawn through the means of eight separate titrations of normal (nonparked) T cells.

antigen-stimulated survival of the relevant lymphocyte subpopulation and the concomitant disappearance of cells lacking specificity for the selecting alloantigens (6 and footnote 1). Thus, if SRBC-reactive T cells belonged to a different subset, they would not be favored for survival during the selection of specific alloantigen-reactive T cells, and they should be depleted from the cultures.

One possible objection to the interpretation of these studies is concerned with the "purity" achieved in the positively selected alloantigen-reactive populations in terms of the extent of contamination by irrelevant bystanders. At present, it is not possible to refute this objection formally, and it is of particular concern to us in view of the finding that positively selected T populations having 10-fold-enriched GVH reactivity to one haplotype also possess normal GVH reactivity for third party haplotypes (6 and footnote 1). However, we have provisionally attributed this third party reactivity to shared alloantigenic determinants rather than to irrelevant bystanders, principally because third party haplotype reactivity (*a*) is quantitatively normal in local GVH assays, i.e. equal to that present in a normal, unselected population of T cells which are, by definition, all bystanders (6); (*b*) shows accelerated kinetics in analytical MLI reactions, thus suggesting that the cells responsible were previously stimulated by the original priming haplotype alloantigens[1]; and (*c*) is markedly diminished in terms of the potential to develop cytotoxic effector cells specific for third party targets, suggesting that the selection against cytotoxic effector cell precursors with third party specificity is complete in this system.[2]

This interpretation of normal third party haplotype reactivity in positively selected populations must be considered as provisional at present, especially in view of the recent demonstrations by Binz and Wigzell of complete positive selection, with no third party reactivity, achieved mechanically, rather than functionally, with affinity column separation of alloantigen-reactive cells with anti-idiotypic sera (7).

Nevertheless, if bystanders are to be provisionally discounted, then the present experiments rule out the possibility that a minority subpopulation of T cells is responsible for nonhistocompatibility antigen recognition. The most direct explanation then is that T

[2] Wilson, D. B., A. Marshak, G. Pierson, and J. C. Howard. Submitted for publication.

cells have more than a single specific reactivity. Thus, a given T cell reactive to one or another of the histocompatibility alloantigen determinants of the species would also recognize other determinants of the antigenic universe due to (*a*) conformational similarities recognized by a single receptor combining site, (*b*) the fortuitous association of multiple combining sites with different specificities on the same receptor molecule (14), or (*c*) the presence of different receptor molecules with different specificities on the same T cell, a suggestion that has been made before (15).

Whether or not alloantigen-reactive T cells also possess specificity for conformationally dissimilar determinants of nonhistocompatibility antigens is a crucial question. If so, this would help to resolve difficulties encountered in attempts to account for the high frequency of alloantigen-reactive T cells and the disproportionate intensity of alloaggressive phenomena within the constraints and predictions of the clonal selection hypothesis. It would also raise important questions of the genetic and structural basis of the association of histocompatibility and nonhistocompatibility antigen receptors present on T cells.

Summary

These studies show that positively selected T-cell populations, having enriched reactivity in the mixed lymphocyte interaction and the graft-versus-host reaction to strong alloantigens of a chosen major histocompatibility complex haplotype, also possess helper activity which is quantitatively normal in the generation of primary antibody responses to sheep red blood cells in vitro. Such positively selected populations give a linear dose plaque-forming cells response curve indistinguishable from that seen with normal unselected T-cell populations. These findings imply that T cells reactive to histocompatibility antigens also react to conventional antigens, and the possibility is raised that they may do so by some recognition mechanism involving multiple specificities.

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