COLLABORATION OF ALLOGENEIC T AND B LYMPHOCYTES IN THE PRIMARY ANTIBODY RESPONSE TO SHEEP ERYTHROCYTES IN VITRO*

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A variety of studies have demonstrated the requirement for collaborative interactions between thymus-derived (T) and bone marrow-derived (B) lymphocytes in the humoral immune response to thymus-dependent antigens (1, 2). Although the precise role of T cells in T-B cooperation is not yet clear, it is known that T cells produce factors upon stimulation with antigens or with allogeneic cells that have direct effects on B cells (3–5). Recently, Taussig and his colleagues described one such factor prepared from "educated" mouse thymocytes which has the ability to totally replace the requirement for T cells in the stimulation of antibody responses to the thymus-dependent antigen, poly(Tyr,Glu)-polyDLAla--polyLys [(T,G)-A--L],[1] in vivo. Subsequent studies showed this factor to be antigen-specific and equally effective in promoting antibody responses by both syngeneic and allogeneic B cells (6, 7).

These findings are in contrast to the recent demonstrations of Katz et al. (8, 9) and of Kindred and Shreffler (10) that effective physiologic T-B collaboration occurs only when the participating T and B cells share surface determinants specified by the major histocompatibility complex (MHC). Allogeneic T and B populations differing with respect to *I*-region determinants of the MHC failed to collaborate, while effective T-B interactions were observed with the use of parental strain and F_1 hybrid T-B cell mixtures.

The experiments reported here provide results which suggest that the failure of effective collaboration in humoral immune responses with allogeneic T- and B-cell mixtures might be a secondary consequence of overriding allogeneic interactions between the participating B and T populations. This possibility was tested directly in T- and B-cell recombination experiments with rat thoracic duct lymphocytes (TDL) in modified Mishell-Dutton cultures stimulated with sheep erythrocytes (SRBC). The results show that B cells can collaborate with allogeneic T cells as effectively as with syngeneic T cells when the allogeneic T population is specifically depleted of immune reactivity to alloantigens of the B-cell donor.

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[1] *Abbreviations used in this paper:* AUG, August; L, Lewis; MHC, major histocompatibility complex; MLI, mixed lymphocyte interaction; PFC, plaque-forming cells; TDL, thoracic duct lymphocytes; (T, G)-A--L; poly(Tyr,Glu)-polyDLAla--polyLys.

Materials and Methods

B and T Cells. B cells were derived from TDL obtained by cannulation of August 28807 (AUG; Ag-B5) B-rat donors that had been thymectomized, irradiated, and reconstituted with small numbers of T-cell-deficient syngeneic marrow cells (11). T cells were obtained from TDL of normal AUG, Lewis (L; Ag-B1) or from (AUG/L)F_1 hybrid donors; B cells were removed from these TDL suspensions by treatment with cytotoxic antisera specific for rat peripheral B lymphocytes followed by guinea pig complement (12). Negatively selected L TDL populations, specifically depleted of reactivity to AUG alloantigens were prepared by passing L TDL through irradiated (450 R) (AUG/L)F_1 "filter" rats according to procedures established by Ford and Atkins (13). They were subsequently treated with anti-B serum and complement.

Cultures. AUG strain B cells (0.5×10^6) and varying numbers of syngeneic, allogeneic (L), negatively selected allogeneic (L_{AUG-}), or semiallogeneic (AUG/L)F_1 T cells were stimulated with SRBC (10^6) in flat-bottom microtiter plates (Linbro Chemical Co., New Haven, Conn.). Each well contained 0.3 ml culture medium consisting of RPMI 1640 medium (Grand Island Biological Co., Grand Island, N.Y.) with 10% vol/vol fetal calf serum (Reheis Chemical Co., Chicago, Ill.), 5×10^{-5} M 2-mercaptoethanol (Sigma Chemical Co., St. Louis, Mo.), 100 μl/ml "PenStrep" (GIBCO), and 0.25 mg/ml glutamine (GIBCO). The cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2—95% air. After 5.5 days of incubation the number of direct plaque-forming cells (PFC) was determined by the modified Jerne PFC assay on agar-coated slides (14, 15).

Reactivity of Negatively Selected TDL. Responsiveness of negatively selected TDL populations to alloantigens was assessed in multipoint popliteal lymph node weight graft-vs.-host (GVH) assays (16) and in mixed lymphocyte interactions (MLI) stimulated with specific and third party alloantigens according to the procedures described extensively elsewhere (footnote 2, 17). Fig. 1 shows the almost complete absence of reactivity of negatively selected L TDL populations for AUG strain alloantigens in the GVH and MLI assays and the persistence of normal responsiveness to third party alloantigens.

Results

Fig. 2 shows the PFC responses in eight experiments in which increasing numbers of syngeneic (AUG) T cells and a constant number of AUG B cells (0.5×10^6) were stimulated in cultures with SRBC. The number of PFC observed after 5.5 days of culture increased linearly on log-log plots as a function of the number of T cells added between doses of 10^4 and 2×10^5, after which the number of PFC showed a plateau with addition of more T cells.

The following experiments (Fig. 3) assess the effectiveness of normal unselected allogeneic (L), negatively selected allogeneic (L_{AUG-}), and semiallogeneic (AUG/L)F_1 T-cell populations as helper cells in comparison to the "standard" PFC response curve obtained with syngeneic T-B mixtures. As before, AUG B cells (0.5×10^6) were combined with titrated dosages of the various T-cell populations. Controls consisted of the T and B populations cultured separately in the presence of SRBC, and of the various T-B mixtures cultured without SRBC.

The results show the following: (*a*) allogeneic T cells possessing immune competence for the alloantigens of the B-cell donor were markedly different from syngeneic T cells in their helper activity; at high T-cell numbers the PFC responses were depressed while at lower T-cell numbers the responses were augmented. Indeed, PFC responses by B cells in the presence of as few as 10^3 allogeneic T cells were well in excess of background values ($\bar{5}$ PFC/10^6 B cells) from cultures in which SRBC were omitted. (*b*) Negatively selected allogeneic T

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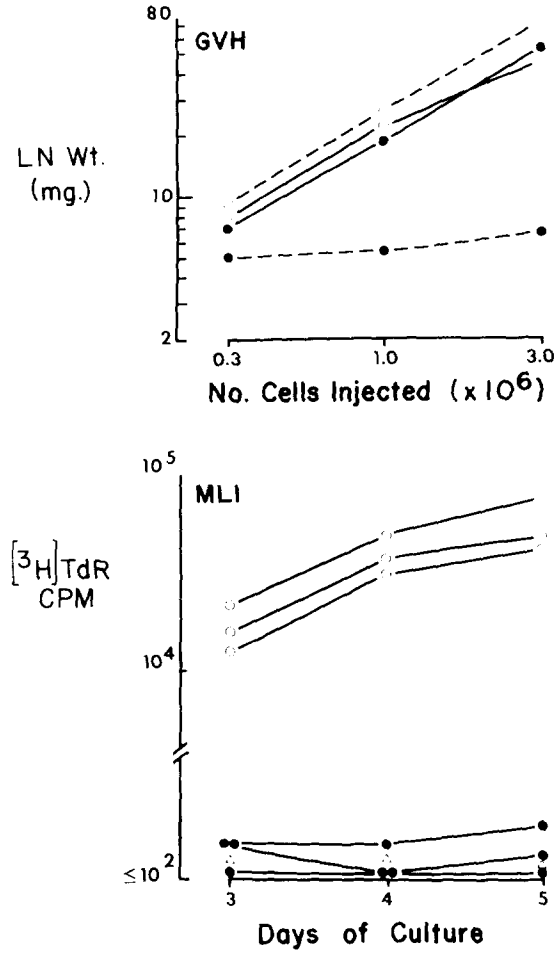

FIG. 1. Reactivity of negatively selected TDL populations (10^9 L TDL) passed through irradiated (450 R) (AUG/L)F_1 "filter" rats. TDL were collected over the succeeding 24-h period and tested in GVH and MLI assays against specific (AUG/L)F_1 and third party (L/DA)F_1 alloantigens.

In the GVH assays, normal L (——) and negatively selected L_{AUG^-} (---) TDL were injected into the rear footpads, at the indicated dosages, of (AUG/L) (●) and (L/DA) (○) recipients. The popliteal lymph nodes were removed and weighed 1 wk later. Each point represents the mean of three such lymph node weights, and the standard errors (not indicated) were less than 20%.

In the MLI, three different negatively selected populations, (L_{AUG^-}), (0.2×10^6 cells/well) were stimulated in microtiter plates for various periods with irradiated (2,000 R) peripheral blood leukocytes (0.2×10^6/well) from specific, (AUG/L)F_1 (●) and from third party, (L/DA) F_1 (○) donors. The cultures received [^{3}H]TdR (0.25 μCi; 6.7 Ci/mmol) 16 h before they were terminated. Background values (Δ) of stimulated cultures did not exceed 100 cpm. The standard errors (not indicated) did not exceed 10% of mean values. Controls consisted of normal, unselected TDL populations which gave responses similar to those indicated for the negatively selected population to third party alloantigens; these curves have been omitted for the sake of clarity.

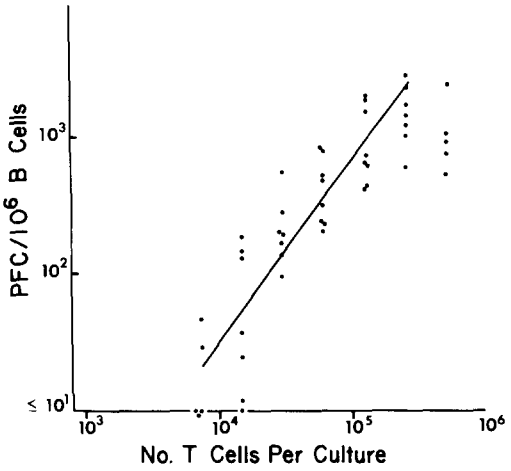

FIG. 2. Cooperative interaction of syngeneic AUG T and B cells derived from rat thoracic duct in the generation of SRBC-specific PFC in culture. Varying numbers of T cells were added to 0.5×10^6 B cells + 10^6 SRBC which were cultured in 0.3 ml RPMI 1640 supplemented with 10% FCS and 5×10^{-5} M 2-ME and assessed for antibody production after 5.5 days of culture. These data show the results of eight different experiments in which the number of PFC/10^6 B cells is plotted as a function of the T cells employed as helpers. The curve represents the regression line fitted by least squares analysis on log transformed data. Each point represents the geometric mean of two-three cultures.

populations, depleted of reactivity to B-cell donor alloantigens, showed helper effects which were quantitatively identical to the dose/response curves of the syngeneic T-B combinations. (*c*) Semiallogeneic (AUG/L)F_1 T cells, genetically unreactive to B-cell alloantigens, also gave PFC responses identical to the syngeneic cultures. Throughout these experiments, PFC were complement-dependent and required the combined presence of T and B cells as well as SRBC for their development in culture.

Discussion

This study provides a direct quantitative comparison of the helper effects of syngeneic and allogeneic rat T cells in the stimulation of SRBC-specific PFC responses by B cells in culture. These comparisons are based on dose/response plots of the number of PFC generated vs. the number of T cells employed as helpers. The principal findings of this study are: (*a*) allogeneic T-cell populations, possessing competence for the alloantigens present on B cells, showed significant differences in helper activity (Fig. 2) in comparison to cultures of syngeneic T-B mixtures (Fig. 1); in cultures with B cells and high dosages of allogeneic T cells, fewer PFC were generated, while at low T-cell doses PFC responses were augmented. (*b*) With the use of negatively selected populations of allogeneic helper T cells, specifically deprived of reactivity to B-cell alloantigens (Fig. 3), the PFC responses were quantitatively identical to that observed with syngeneic T-B mixtures (Fig. 2).

Previous reports by Katz et al. have indicated the inability of carrier-primed

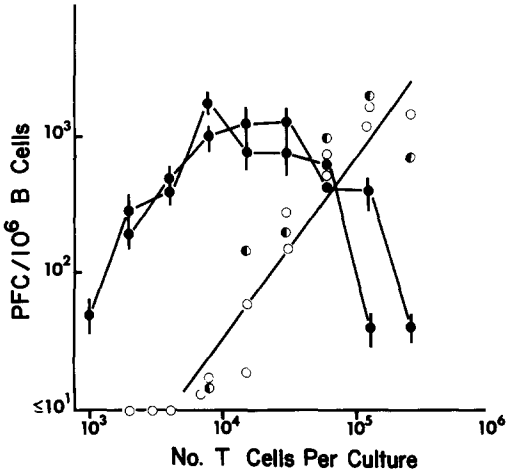

FIG. 3. Cooperative interaction of normal, unselected allogeneic Lewis (●), negatively selected allogeneic L_{AUG^-} (○), and of (AUG/L)F_1 T cells (◐) with AUG B cells in the stimulation of SRBC specific PFC in culture. The straight line represents the regression curve of syngeneic AUG T-B mixtures (Fig. 2) and is presented here as a reference against which the test T-B mixtures are compared. Varying numbers of T cells were added to 0.5×10^6 AUG B cells which were cultured for 5.5 days at 37°C and then assayed for PFC generation.

T-cell populations to provide adequate helper effects for hapten-primed allogeneic B cells in F_1 mice subsequently boosted with specific hapten-carrier conjugates (8). These studies also demonstrated effective cell-cell collaboration of the participating T and B populations sharing an MHC haplotype (use of parental and F_1 donors). The interpretation offered for their findings was that identity of at least a single MHC haplotype was required for effective T-B interactions, and that H-gene products of the MHC locus constitute a basic physiologic control mechanism underlying cell-cell interactions in the immune response. A subsequent study mapped these relevant MHC genes in the *I* region of the MHC (18).

A prediction of this hypothesis is that allogeneic T cells, prepared in such a manner that they are unreactive to B-cell alloantigens, would still not collaborate effectively with B cells since they do not possess a B-cell-specific MHC haplotype required for such collaboration. Furthermore, reduced competence in the T-cell population for B-cell alloantigens would only have the consequence of diminishing the level of antibody known to be produced when allogeneic effects prevail (19). The experiments reported here fail to support this general hypothesis. First, while PFC responses in allogeneic T-B cultures were decreased relative to normal, this was seen only at high T-cell dosages; lower dosages of T cells augmented the PFC responses. Secondly, negatively selected allogeneic T-cell populations lacking competence for the alloantigens of the B-cell donor, displayed helper effects quantitatively identical to that of syngeneic T cells. Thus, the suppressive effects with high numbers of T cells and the augmented responses at low numbers of T cells in allogeneic T-B combinations appear to be a secondary consequence of allogeneic reactions by immunocompetent T cells directed to B-cell alloantigens. Consequently, there seems to be no requirement

for shared MHC haplotypes among rat T and B cells from thoracic duct lymph in direct PFC responses to SRBC in culture.

Unfortunately, these experiments do not provide a satisfactory accounting for the effective collaboration of parental T and F_1B cells demonstrated by Katz et al. (8) under the same in vivo conditions where allogeneic T- and B-cell combinations failed to interact. This inconsistency may indicate the occurrence of other unanticipated allogeneic interactions among the participating cell populations.

The present findings provide support for the suggestion of Bechtol et al. (20) that allogeneic T and B cells interact in vivo in tetraparental mice immunized with (T,G)-A--L. In these studies tetraparental mice, possessing T and B cells of both high responder and low responder parental genotype and differing also by an immunoglobulin allotype, were shown to produce significant titers of anti-(T,G)-A--L antibody of nonresponder allotype, presumably through a cooperative interaction of low responder B cells and high responder allogeneic T cells, both having specificity for the immunizing antigen. However, it is not possible to formally eliminate the alternative possibility that an ongoing allogeneic response by T cells occurred at a level sufficient to bypass the requirement for T-cell activation to carrier determinants of the immunizing antigens in these animals.

Some of the findings in the present experiments are of particular relevance to considerations of the mechanism of B-cell triggering. First, unlike most studies which employ splenic or lymph node cell suspensions, the participating T and B cells employed in these studies are lymphocytes obtained from the thoracic duct, a fact that tends to minimize, but does not totally exclude, the participation of macrophages in the generation of SRBC specific PFC responses.

Secondly, the minimal number of T cells in the syngeneic and negatively selected allogeneic T populations, which includes a frequency of SRBC antigen-specific T cells sufficient to support detectable PFC responses by 0.5×10^6 B cells, can be estimated to be approximately 10^4. The finding that as few as 10^3 normal, unselected allogeneic T cells will support detectable B-cell responses makes it unlikely that the participating T population in this instance includes T cells with specificity for SRBC determinants and equally unlikely, if T-cell factors are involved, that they would possess such specificity.

Finally, the PFC responses observed in these experiments in both the syngeneic and allogeneic cultures depends upon the combined presence of B and T cells and antigen as well. It can therefore be presumed that B-cell triggering in this system requires the presence of antigen and the opportunity for cell-cell interactions; although both are necessary, neither alone is sufficient, a conclusion which is pertinent to current considerations (21, 22) of one vs. two signal models of B-cell activation.

Summary

This study provides a direct quantitative comparison of the helper effects of allogeneic and syngeneic rat T cells in the production of direct SRBC plaque-forming cell (PFC) responses by B cells in culture. In syngeneic T-B combinations, log-log plots of the number of PFC generated after 5.5 days in culture vs. the number of T cells employed as helpers showed a linear response between 10^4

and 2.5×10^5 T cells added. Allogeneic T-B combinations, in which the T cells possess the capacity for reactivity to major alloantigens of the B-cell donor, showed a different dose/response relationship in which PFC responses were decreased at high T/B ratios and augmented at low T/B ratios. In this system, responses were detected with as few as 10^3 allogeneic T cells. Use of negatively selected allogeneic T populations, specifically depleted of mixed lymphocyte interaction (MLI) and graft-vs.-host reactivity for B-cell alloantigens, as helpers gave dose/response curves quantitatively identical to responses with syngeneic T-B combinations and also with F_1 T-cell parental B-cell combinations.

These data indicate that rat T and B cells need not share a major histocompatibility complex haplotype in order to collaborate effectively in a primary direct PFC response to SRBC in culture. In addition, the PFC response required the combined presence of T and B cells as well as antigen in the cultures, a finding consistent with the two signal model of B-cell activation. Finally, the dose/response data obtained suggest the possibility that although SRBC antigen is required in the cultures helper activity with low numbers of normal allogeneic T cells may not depend on T cells having specificity for this antigen.

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