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Detection of a Novel Binding Site for T Cell Derived Antigen Binding Proteins on Thymic Epithelial Cell Surfaces

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Summary

The presence of helper T cells that do not recognize major histocompatibility complex encoded antigenic determinants but rather are specific for self idiotypic determinants led us to search for a mechanism by which such cells might be influenced by idiotype expressed on the surfaces of thymic epithelial cells. Thymic epithelial cells were cultured and characterized as being rich in surface Ia antigens and keratin positive. Such cells do not have conventional Fc receptors for immunoglobulin. However, they will bind specifically to T cell derived antigen binding molecules (T-ABMs). This binding could be detected either by staining with specific anti-T-ABM antisera or by subsequent hapten-specific antigen binding. Using anti-T-ABM antisera, it could also be shown that normal thymocytes transfer antigen binding molecules to thymic epithelial cell surfaces in vitro. *These studies indicate that thymic epithelial cells can express passively acquired, T cell derived idiotype on their surface. This surface bound, T cell derived idiotype is proposed to play a role in the selection of anti-self idiotypic T cells.*

Keywords: Helper T cell, idiotype, Fc receptor, T cell receptor

Abbreviations

BSA = Bovine serum albumin
DNP = 2,4-dinitrophenyl
Ig = Immunoglobulin
MHC = Major histocompatibility complex
T-ABM = T cell derived antigen binding molecule
TE = Thymic epithelium
TNP = 2,4,6-trinitrophenyl

Introduction

T lymphocyte responses have been shown to involve recognition both of foreign antigen and of self major histocompatibility complex (MHC) encoded antigens in many instances[1]. Studies of radiation induced bone marrow chimeras have strongly suggested a selective role for MHC encoded antigen bearing radio-resistant portions of the thymus in the development of self MHC recognition by mature T cells[1-3]. Recently, a distinct set of helper T cells has been characterized which have specificity for unique determinants on self immunoglobulin (Ig) molecules, or idiotypes[4-8]. Such helper T cells are antigen-specific[4,5], but do not demonstrate MHC restriction in their response to antigen or their interaction with B cells[6-8]. By analogy to MHC-recognizing T cells, one of us (K.B.) has proposed that these idiotype-recognizing T cells might be selected for their ability to recognize self idiotypic determinants during their development within the thymus[9]. It seems unlikely that non-lymphoid, radio-resistant components of the thy-

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mus would bear endogenously produced Ig idiotypes, a property hitherto reserved to lymphocytes. Instead, thymic reticulo-epithelial cells might bear idiotypic determinants acquired passively from adjacent lymphoid cells, in this case thymocytes.

Because of the postulated role of non-lymphoid thymic elements in T cell development, several laboratories, including our own, have examined thymic reticulo-epithelial elements *in vitro*[10-12]. A simple technique has been developed in our laboratory for culturing murine thymic components[12]. These cultures contain two major cell types: phagocytic cells with conventional Ig-binding Fc receptors; and epithelial cells that lack such receptors. The latter cells are large, have a dendritic form, and contain tonofilaments and intracytoplasmic keratin; we shall therefore refer to these cells as thymic epithelium (TE). TE cells have readily detectable cell surface Ia antigens[12]. Since T cell recognition of Ia antigens plays a critical role in T-B and T-Macrophage interactions[13-15], their presence in large amounts on TE cells is consistent with the postulated role of TE in T cell development.

The proposal that helper T cells bearing receptors for self idiotypic determinants are selected by recognition of T cell idiotypic determinants in the thymus is predicated on two findings: first, that anti-self idiotypic T cells appear to be primarily specific for the predominant or germ line encoded idiotypes of a given strain[4-9,16,17], and second, that T cells bear receptors for antigen that carry mainly or entirely these same germ line idiotypic specificities[18-20]. Thus, if anti-self idiotypic T cells are selectively activated by idiotypes encountered during intra-thymic development, they would tend to be committed to recognize the germ line idiotypes expressed by T cells, which indeed seems to be the case[9].

In the present experiments, these concepts led us to examine cultured TE cells for cell surface receptors for T cell derived antigen binding molecules (T-ABMs). We find that TE indeed bears a cell surface binding site for T-ABMs, that such T-ABMs bind to TE in such a way that their antigen-binding site is exposed on the TE cell surface, and that normal thymocytes rapidly transfer T-ABMs to TE cells *in vitro*. Thus, these experiments demonstrate a mechanism by which T cells could be selected on TE cell surfaces for possession of anti-self idiotypic receptors.

Materials and Methods

Thymic epithelial cultures

The preparation of TE cultures from 3 week old mouse thymuses is described elsewhere[12]. Briefly, thymuses were gently minced, washed by unit gravity sedimentation, and cultured in petri dishes in modified Dulbecco's medium. Cultures were fed periodically using decreasing concentrations of fetal calf serum. After 3–6 weeks of culture, the cells were used in the experiments.

T Cell derived antigen binding molecules

T-ABMs were obtained from the supernatants of spleen and lymph node cultures from mice immunized with TNP or DNP to produce specific suppressor factors, and affinity purified on TNP or DNP sepharose as previously described[21,22].

Anti-T-ABM antisera

Anti-T-ABM antibody was prepared by immunizing rabbits with affinity purified T-ABMs as previously described[22]. Such antisera react with a wide variety of T cell derived antigen binding molecules but do not react with Ig[22,23].

Staining TE

Staining was carried out at 37°C as previously described[12]. Cultures were stained in petri dishes and washed three times by decantation after each component was added. Staining times were 30'. After staining, cultures were examined by incident beam fluorescence microscopy. TNP_{12}-BSA, affinity-purified guinea pig anti-TNP coupled with fluorescein, and sheep anti-rabbit Ig coupled with fluorescein were prepared by standard techniques.

Results

Thymic epithelial cells bind T cell derived antigen binding molecules

In order to determine whether TE cells could bind T-ABMs, cultured TE were incubated with various preparations of T-ABMs. These T-ABMs were specific for the haptens TNP and DNP, and they were purified by affinity chromatography on TNP or DNP sepharose columns. Binding of T-ABMs was detected with rabbit anti-T-ABM antibody which stains most peripheral T cells but not B cells. Such antibodies also precipitate a 68,000d molecule from T cell surface membranes, but do not react with Ig. As seen in Table 1 and Figure 1, TE cells could be shown to bind T-ABMs by staining with rabbit anti-T-ABM followed by fluorescein-conjugated sheep anti-rabbit Ig. In these experiments, both macrophages and TE cells stain brightly, the latter with a beaded pattern along the dendritic processes. Epithelial cells not incubated with T-ABMs did not stain, indicating that such cells do not bear T-ABMs after 3–6 weeks in culture.

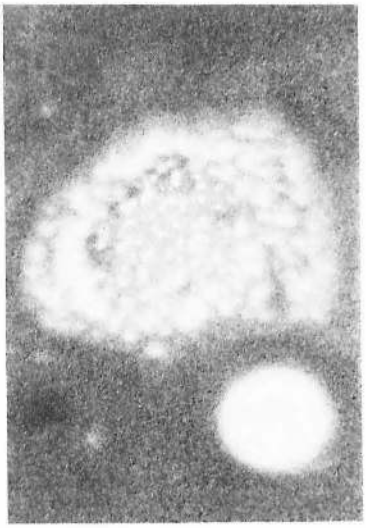

Fig. 1: Thymic epithelial cell (top) and macrophage (bottom) stained with T-ABM followed by anti-T-ABM antibody and FITC-sheep-anti-rabbit Ig. (500x)

Thymic epithelial cell binding of T-ABMs is not inhibited by immune complexes

Macrophages in thymic explant cultures bear conventional Fc receptors for Ig. It thus seemed possible that we could prevent uptake of T-ABMs by the macrophages by preincubating the cultures with aggregated Ig or antibody coated erythrocytes prior to adding the T-ABMs. *Ptak* et al.[24] had reported such inhibition in functional assays. Preincubation of the cultures with antibody coated erythrocytes had no effect on staining of the epithelial cells (Fig. 2), but significantly reduced staining of the macrophage, suggesting that macrophage but not epithelial cell binding of T-ABMs is due to conventional Fc receptors for Ig, and confirming previous functional studies[24].

Thymic epithelial cells bind T-ABMs with their antigen binding sites exposed

If the postulated role of TE cells in presenting T-ABMs to anti-idiotypic T cells is valid, then the T-ABMs must be bound such that their idiotypic de-

Table 1 Binding of T-cell derived antigen binding molecules to thymic epithelium

First stain	Second stain: rabbit anti-T-ABMs (1:40)	Third stain: FITC-sheep anti-rabbit Ig (1:20)	Cell surface fluorescence of thymic epithelial cells
DNP-specific T-ABMs	+	+	++++
TNP-specific T-ABMs	+	+	++++
Affinity-purified TNP-specific T-ABMs	+	+	++++
–	+	+	–
–	–	+	–

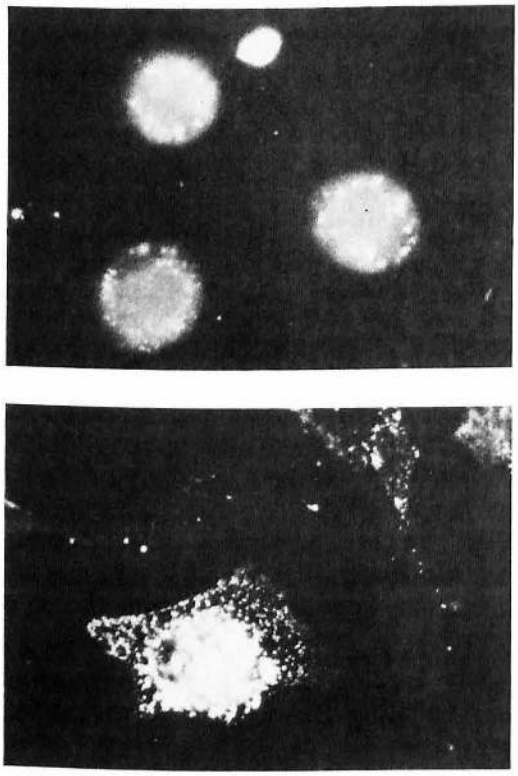

Fig. 2: Thymic explant cultures preincubated with antibody-coated sheep erythrocytes. Free erythrocytes removed by gentle washing. Culture stained as in Figure 1 to detect subsequent binding of T-ABM. Top panel, thymic epithelium cells still bind T-ABM. Bottom panel, T-ABM binding by macrophages is inhibited.

terminants are exposed on the TE cell surface. Since idiotype is associated with the antigen binding site, we asked whether T-ABMs specific for TNP could bind TNP-BSA to the TE cell surface. Bound TNP-BSA was detected by staining with fluorescein conjugated, affinity purified guinea pig anti-TNP antibody. The staining obtained was identical to that in Figure 1, and the results are summarized in Table 2. This demonstrates that T-ABMs bind to TE such that their antigen binding site, and hence their idiotypic determinants are exposed on the cell surface. Thus, the T-ABM binding site on the TE must interact with the non-antigen binding portion of the T-ABM. These experiments demonstrate that TE cells have a specialized acceptor site for T-ABMs, analogous to but distinct from conventional Fc receptors. T-ABMs bound to this site have their antigen-binding (idiotypic) site exposed on the epithelial cell surface.

Thymic epithelial cells can acquire T-ABMs from normal, syngeneic thymocytes

The thymic epithelial cultures used in these experiments were all tested three to six weeks after they were established, at a time of optimal epithelial cell growth. Thymocytes in these cultures degenerate during the first week, and are removed during media changes[12]. To determine whether thymocytes can transfer their cell surface antigen binding molecules to TE cells in culture, fresh syngeneic thymocytes were incubated with TE cultures for 24 hours. Lymphocytes were removed by serial washings and the cultures were stained with anti-T-ABM and fluorecein-anti-rabbit Ig as in Table 1. The staining again was identical to that in Figure 1, while cultures not given thymocytes did not show staining, strongly suggesting that acceptor sites on TE cells can bind to T cell receptor-like molecules produced locally by thymocytes.

Table 2 T-ABMs bound to thymic epithelium can bind antigen

First stain	Second stain: TNP-BSA (100 µg/ml)	Third stain: FITC-anti TNP antibody (1:20)	Cell surface fluorescence of thymic epithelial cells
Affinity-purified TNP-specific T-ABM	+	+	++++
Affinity-purified TNP-specific T-ABM	−	+	−
−	+	+	−
−	−	+	−

Absence of genetic restrictions in T-ABM binding to TE cells

To determine whether T-ABM binding to TE cells showed genetic restrictions, CBA/J ($H\text{-}2^k$, Ig^i) anti-TNP-T-ABMs were tested for binding to TE from strains having $H\text{-}2^{k,d,b}$ and $Ig^{a,b,c,d,e}$. All cultures tested did bind the T-ABMs as demonstrated by staining with anti-T-ABM antibody. Thus, the TE acceptor site for T-ABMs would appear to be unaffected by products of MHC or Igh genes, as is true of conventional Fc-Ig receptors as well.

Discussion

These experiments demonstrate that thymic epithelial cells have on their surfaces specialized binding sites for T cell derived antigen binding molecules. Such molecules are bound with their antigen binding sites, and hence their idiotypic determinants, exposed on the cell surface. Normal, syngeneic thymocytes rapidly transfer T-ABMs to TE cells.

By analogy with the postulated role of TE Ia antigens in the positive selection of thymocytes bearing anti-self Ia receptors[1-3], we would propose that TE cells bearing T cell derived idiotypes would positively select thymocytes bearing receptors specific for these idiotypes[9]. Idiotype-recognizing helper T cells are known to require exposure to self idiotype in the periphery for their effective development and expression[4,5,9]. Again, we would view this as being analogous to the requirement of MHC recognizing T cells for peripheral cells bearing the appropriate MHC antigens for their activation[3]. The rationale for postulating a role for T cell derived idiotype expressed on TE cell surfaces in the selection of idiotype-recognizing helper T cells is as follows: idiotype recognizing helper T cells are specific for dominant idiotypes in the systems where they have been studied, and it is just these idiotypes that are expressed by T cells[18,20]. B cells, by contrast, express not only these dominant idiotypes, but variant idiotypes as well. Since B cell derived idiotype clearly plays an essential role in the development of idiotype recognizing helper T cells[4,5], selection for only those T cells bearing receptors specific for the dominant idiotype must exist independent of the role played by B cell derived idiotype. Thus, it was proposed that T cell derived idiotype would select anti-idiotypic T cells in the thymus. Since selection of MHC recognizing T cells in the thymus involves T cell contact with TE rather than with T cells (see 1 for discussion), it has been proposed that idiotype expressed on TE surfaces plays this critical selective role in the case of idiotype-recognizing helper T cells. We are currently testing this hypothesis in functional assays.

The acceptor sites for T cell derived antigen binding molecules may play roles different from those that initially led us to study them. Functional studies must determine the roles played by such sites. The present experiments do demonstrate the existence of sites on TE that bind T-ABMs with their antigen binding site, and hence idiotype, exposed on the TE cell surface. They are thus consistent with the hypothesis that T cell derived idiotype on the surface of TE cells plays a role in selecting T cells with specificity for these self idiotypes during ontogeny.

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