CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY 30, 227–232 (1984)

Distinctive Immunological Properties of Cultured Murine Thymic Epithelial Cells

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Skin painting with chemically reactive haptens induces a hapten-specific state of hypersensitivity that is long lasting and can be transferred to unirradiated recipient mice. A similar state of hapten-specific contact sensitivity can be induced by intravenous immunization with hapten-conjugated cells. Thus far, only two cell types have been described that can perform this function: Langerhans cells of the skin, and splenic dendritic cells. All other types, coupled with hapten, induce either tolerance or a short-lived state of contact hypersensitivity that is readily suppressed, and cannot be transferred to normal recipients. In the present experiments, it was demonstrated that culture-enriched, hapten-coupled thymic epithelial cells can also induce a state of stable contact hypersensitivity identical to that induced by skin painting. This provides evidence that thymic epithelial cells have distinctive properties as antigen-presenting cells *in vivo*. The relationship of this finding to the postulated role of thymic epithelium in T-cell development is discussed.

INTRODUCTION

A state of contact hypersensitivity, an *in vivo* measure of T-cell function, can be induced to chemically reactive haptens by several modes of immunization. The classical immunizing procedure is to apply the chemical reactant to the skin. The immune response that ensues has certain distinctive features: (1) it is long lived; (2) it can be adoptively transferred into normal untreated mice; and (3) it is resistant to signals from concomitantly activated suppressor cells (1). Contact sensitivity can also be produced by injecting certain types of syngeneic cells coupled with the contact antigen intravenously. Two types of naturally occurring cells have been found to induce, by this means, a state of contact sensitivity that resembles the one induced by skin painting: the Langerhans cells of the skin (2) and splenic dendritic cells (3). In fact, it is likely that the distinctive features of classically induced contact sensitivity result at least in part from the *in situ* conjugation of the contactant to Langerhans cells in the skin. It is thought that the ability of Langerhans cells and dendritic cells to induce contact sensitivity is

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0090-1229/84 $1.50

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related to the presence of cell surface antigens encoded in the I region of the major histocompatibility complex (Ia antigens). However, other types of Ia^+ cells (splenic B cells, peritoneal exudate cells) coupled with hapten induce a form of contact hypersensitivity that does not share the three distinctive characteristics listed above (4). Under these conditions, the immune response is easily suppressed, and cannot be adoptively transferred unless immune suppression in the recipients is compromised. Thus, hapten-coupled Langerhans or dendritic cells appear to induce suppressor-resistant effector cells, while hapten coupled to other cell types does not. Since thymic epithelial cells, like Langerhans cells and dendritic cells, have a dendritic morphology and express high levels of Ia antigens, and because such cells have been postulated to play a central role in the selection and expansion of self-Ia-recognizing T cells of the helper type during intrathymic maturation, we tested whether hapten-coupled cultured thymic epithelial cells enriched by selective culture (5) could also induce suppressor-resistant contact sensitivity. Their ability to do so suggests a functional homology between these morphologically similar cell types and may have important implications for the *in situ* functioning of these Ia-bearing epithelial cells in the thymus.

MATERIALS AND METHODS

Mice. All experiments were carried out using CBA/J mice purchased from Jackson Laboratory, Bar Harbor, Maine. Mice were 8–12 weeks old, except for thymus donors, who were 3 weeks of age when their thymuses were put into culture.

Antigens and immunization. Trinitrochlorobenzene was purchased from Eastman Kodak, Rochester, N.Y.; it was dissolved in olive oil as a 1% solution and applied to shaved abdominal skin to induce contact sensitization (positive control). Trinitrobenzenesulfonic acid (Eastman) was used to couple cells and was also injected as a solution in phosphate-buffered saline (PBS) into mice in order to induce tolerance; the dosage was 4 mg/mouse, injected via the retroorbital plexus of veins.

Assessment of contact sensitivity. Mice were challenged with 10 μl of 1% trinitrochlorobenzene applied to either side of the ear 7 or 21 days after immunization, and the increment in ear thickness was determined with an engineer's micrometer 18–24 hr later.

Preparation of cells. The establishment of thymic epithelial cell cultures have been detailed elsewhere (5). Briefly, thymi were removed from CBA/J mice (Jackson Laboratories) less than 3 weeks of age, finely minced, and placed in Dulbecco's modified Eagle's medium (Gibco, Grand Island, N.Y.), 0.15% in sodium bicarbonate, containing 1 m*M* sodium pyruvate (Flow, Dublin, Va.), and 2 m*M* Hepes (Gibco) plus 1% antibiotic/antimycotic solution (Gibco). This base medium was initially supplemented with 30% fetal calf serum which was gradually reduced to 10% over 3 weeks in culture. Explants were cultured in 25-cm^2 flasks (Falcon, Oxnard, Calif.) at 37°C with 5% CO_2 in air at 100% humidity. Using this technique, thymocytes and fibroblasts were virtually absent from the culture at

7 days. Greater than 70% of the remaining cells were epithelial in nature, with macrophages representing 10–30% of the contaminating cells.

Confluent cultures were washed with PBS in flasks and were trinitrophenylated by the addition of 2.5 m*M* trinitrobenzenesulfonic acid (Eastman) in phosphate-buffered saline (pH 7.3). The reaction was allowed to continue for 10 min at 37°C after which medium containing 10% fetal calf serum was added to competitively inhibit further haptenation of the cells. They were removed from the surface of the flask by curettage and vigorous pipetting and washed several times in medium containing fetal calf serum. When indicated, cells were then irradiated with 1000 rad while on ice. Following these procedures, viable cells were counted using trypan blue exclusion as a criterion. Cells were injected intravenously into the tail of syngeneic CBA/J males, 6–8 weeks old.

Thymic macrophages. Thymic macrophages were enriched by flotation of cells in bovine plasma albumin (BPA) (Armour Pharmaceutical) using the method described by Steinman *et al.* for isolation of splenic dendritic cells (6). Briefly, a suspension of cells was prepared from mechanically disrupted thymi, pelleted, and resuspended in dense PBA. This was gently overlaid with light PBA and centrifuged at 8000 rpm for 20 min in the cold. The cells at the interface were washed one time and rosetted on ice with sheep red blood cells (SRBC) sensitized with hyperimmune rabbit anti-SRBC antibody at a ratio of 50:1. The rosetted cells were then suspended in light BPA and layered onto dense BPA. After centrifuging at 8000 rpm for 20 min in the cold, the pelleted Fc receptor-positive thymic macrophages were recovered and red cells were lysed with 0.83% Tris–ammonium chloride before trinitrophenylation.

Transfer of contact hypersensitivity. Spleen cells from immune mice were teased into a single-cell suspension 21 days after priming. Cells (5×10^7) were injected iv via the tail vein into naive recipients. Contact sensitivity was elicited immediately after cell transfer.

RESULTS

Hapten-Coupled Culture-Enriched Thymic Epithelial Cells Induce Contact Sensitization

To determine the capacity of thymic cells to induce immunity or tolerance for contact hypersensitivity, cells that had been established in culture (5) were trinitrophenylated and injected intravenously into syngeneic mice. A contact sensitivity response could be elicited at both 7 and 21 days after immunization (Table 1), demonstrating the persistence of the immunity induced. This ability of haptenated thymic epithelial cells to immunize for long-lived contact hypersensitivity was also resistant to X irradiation of the cultivated haptenated cells with 1000 rad.

Hapten-Coupled Thymic Epithelial Cells Induce Suppressor-Resistant Contact Sensitivity

We next determined whether the cultured hapten-coupled thymic epithelial cells could induce immunity if suppression was activated simultaneously by intrave-

TABLE 1
INDUCTION OF CONTACT SENSITIVITY BY INTRAVENOUS INJECTION OF TNP-THYMIC EPITHELIAL CELLS: RADIATION RESISTANCE[a]

Group	Cell No.	Treatment	% Increase ear swelling ± SD	
			Day 7	Day 21
A	3×10^5	None	20.8 ± 3.7	17.5 ± 3.3
B	6×10^5	1000 rad	N.D.	20.2 ± 1.2
C	3×10^5	1000 rad	N.D.	30.6 ± 1.8
D	1.5×10^5	1000 rad	N.D.	27.2 ± 4.0
E	None	None	8.8 ± 2.3	6.2 ± 1.2

[a] Ear swelling responses in mice injected 7 or 21 days previously with TNP-coupled cultured syngeneic thymic epithelial cells upon challenge with 10 μl of 1% trinitrochlorobenzene in olive oil applied to each side of the ear. Increment in ear thickness was measured with an engineer's micrometer at 18–24 hr. N.D., not done. $P < 0.05$ for Groups A, B, C, and D vs E on both Day 7 and Day 21. Results were compiled from two independent experiments in which each experimental group consisted of four mice (total of eight mice/point). Typically, we find a 20–30% increment in ear swelling following sensitization by topical application of PCl (2, 3).

nous injection of trinitrobenzenesulfonic acid (TNBS) (3, 4). The results of these experiments (Table 2) show that the induction of immunity by haptenated cultured thymic epithelial cells was insensitive to coadministration of tolerogenic doses of TNBS (A vs B). This capacity was radioresistant (C vs D). In this experiment, there was even a marked tendency for TNBS to augment the immune response induced by haptenated thymic epithelial cells.

Hapten-Coupled Thymic Macrophages Induce Short-Lived, Suppressible Contact Sensitivity

Since thymic macrophages have been shown to act as antigen-presenting cells in several *in vitro* assays of immune function (7, 8) and since these cells represent

TABLE 2
COMPARISON OF ANTIGEN-PRESENTING CAPACITY OF THYMIC EPITHELIAL CELLS AND THYMIC MACROPHAGES: SENSITIVITY TO COADMINISTRATION OF A TOLEROGENIC DOSE OF TNBS[a]

Group	Cells used for Immunization	Treatment	Tolerogen	% Increase ear swelling ± SD at Day 7
A	TNP-TE	None	None	18.6 ± 2.4
B	TNP-TE	None	TNBS	24.5 ± 4.3
C	TNP-TE	1000 rad	None	19.3 ± 3.9
D	TNP-TE	1000 rad	TNBS	32.0 ± 5.5
E	TNP-macrophage	None	None	15.3 ± 3.5
F	TNP-macrophage	None	TNBS	10.3 ± 3.4
G	TNP-macrophage	1000 rad	None	7.0 ± 3.7
H	TNP-macrophage	1000 rad	TNBS	7.7 ± 4.4
I	None	None	None	6.0 ± 3.0
J	None	None	TNBS	6.2 ± 2.4

[a] Mice were injected with 3×10^5 syngeneic cells as noted and, within minutes, 4 mg TNBS was injected iv via the retroorbital plexus. Mice were challenged 7 days later as in Table 1. Three to four mice were used per experimental group.

TABLE 3
THE ABILITY TO ADOPTIVELY TRANSFER CONTACT SENSITIVITY INDUCED BY INTRAVENOUSLY INJECTED TNP-THYMIC EPITHELIAL CELLS[a]

Group	Immunization of transferred cells	% Increase ear swelling ± SD
A	3×10^5 TNP-TE	29.9 ± 2.4
B	None	4.3 ± 1.3

[a] Spleen cells (5×10^7) pooled from three immune donors primed 21 days previously were injected iv into the tail vein of each of four syngeneic recipients. Mice were challenged 24 hr later, and at 24 hr after challenge ear swelling was measured as in Table 1. $P < 0.05$ for Group A vs B.

10–30% of the cells present in these 3-week-old thymus cultures, it was important to determine whether haptenated thymic macrophages alone could account for the production of the suppressor-resistant type of contact sensitivity we had noted. Thus we isolated thymic macrophages, labeled them, and injected them intravenously. The result (Table 2) was the induction of an immune response that could be elicited on Day 7 (E vs I) but this immunity was eliminated by a tolerogenic dose of TNBS (F vs J), was sensitive to irradiation with 1000 rad (G vs I), and was not elicited at rechallenge on Day 21 (data not shown).

Hapten-Coupled Thymic Epithelial Cells Prime for Transfer of Contact Sensitivity to Normal Recipients

A third important measure of the suppressor-resistant immune response induced by specialized presenting cells is its ability to be adoptively transferred into naive untreated recipients (9). We found (Table 3) that as few as 3×10^5 haptenated thymic epithelial cells could induce an immune response that could be adoptively transferred in this manner. These results demonstrate that thymic epithelial cells can present antigen to the immune system *in vivo* to induce a suppressor-resistant state of contact sensitivity as effectively as any heretofore described peripheral antigen-presenting cell.

DISCUSSION

The finding of suppressor-resistant contact sensitivity has been correlated in previous studies with the induction of contrasuppressor T cells; when these cells are deleted from immune T-cell populations, adoptive transfer can only be performed using recipients whose suppressor T cells have been compromised (9). Hence, our studies suggest strongly that haptenated thymic epithelial (TE) cells induce at least two sets of T cells: an effector cell that mediates the contact reaction and a contrasuppressor cell that protects the effector from suppression.

The finding that haptenated TE cells induce these two T-cell activities may have important implications for the role of thymic epithelium in T-lymphocyte development *in situ*. However, the test system used here is clearly an artificial one, and hence the results must be interpreted with caution. While the thymic cortex is rich in dendritic, Ia-antigen-bearing epithelial cells (10), antigens penetrate this region poorly in mice (11). However, we have previously shown that identically cultured thymic epithelial cells can bind readily detectable amounts of

T-cell-derived antigen-binding molecules (12), and one of us (C.A.J.) has argued that these could represent an internal antigenic image of the external antigenic universe, in accord with Jerne's network hypothesis (13). Thus, presentation of antigenic images by thymic epithelial cells to developing thymocytes may well occur *in situ*, and this could play a significant role in expanding developing T cells in the thymic cortex.

Cortical thymocytes are generally found to be either immunologically inert or somewhat suppressive. Such suppression may be critical for the induction of "self-tolerance," an important stage in T-cell development. However, within this suppressive environment, the precursors of effector T cells must somehow develop, and evidence from thymic chimeras suggests that this maturation involves radioresistant, Ia-antigen-bearing cells (14, 15). Thymic epithelial cells are a good candidate for this role, as they are the only Ia-positive cells in thymic cortex. The induction of contrasuppression by thymic epithelial cells *in situ* may be an additional important factor in allowing effector T-cell development. The interplay of suppressor, helper, and contrasuppressor influences in the cortical thymic microenvironment may be critical in establishing a regulatory balance among mature peripheral lymphocytes. Our studies suggest an important role of thymic epithelial cells in the induction of the helper and contrasuppressor components of this T-cell pool.

Clearly, one would like to be able to confirm these suspected physiological activities of thymic epithelium in a less artificial setting. Nevertheless, the finding that cultured thymic epithelial cells have distinctive physiological abilities to present antigens to effector and contrasuppressor T cells is an important piece of evidence that these cells have a unique immunological role in the thymus, presumably for the development of mature T-cell function of the types described.

ACKNOWLEDGMENTS

This work was supported by the Howard Hughes Medical Institute. The authors would like to thank Carol Sanford and Janet Granger for preparation of this manuscript.

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Received April 11, 1983; accepted with revisions July 8, 1983.