Functional Analysis of Ia Antigen-Bearing Keratinocytes: Mixed Skin Lymphocyte Culture Between Ia Antigen-Bearing Pam 212 Cells and Allogeneic and Syngeneic Splenic T Cells

Setsuya Aiba, M.D., and Hachiro Tagami, M.D.
Department of Dermatology, Tohoku University School of Medicine, Sendai, Japan.

Keratinocytes express Ia antigens in various skin disorders, although the biological role of these Ia antigen-bearing (Ia⁺) keratinocytes remains unclear. We induced Ia antigens on Pam 212 murine keratinocyte cell line by interferon-γ (IFN-γ) and using these cells, we performed the mixed skin lymphocyte culture with syngeneic BALB/c or allogeneic C3H/He splenic T cells. Unexpectedly, Pam 212 cells were found to stimulate both syngeneic and allogeneic T cells irrespective of IFN-γ treatment. However, both syngeneic and allogeneic T cells cultured with IFN-γ-treated Pam 212 cells incorporated [³H]thymidine much more actively than those cultured with IFN-γ-untreated Pam 212 cells. This stimulation was not inhibited by monoclonal anti-I-A⁺ antibody. Analysis of the responding T cells demonstrated that the syngeneic T-cell stimulation by IFN-γ-treated Pam 212 cells occurred in both purified Lyt 1⁻ T cells and Lyt 2⁻ T cells. Furthermore, we found that the T cells cultured with the IFN-γ-treated cells were composed of two morphologically different types of cells. Determination of their surface phenotype showed that the small cell population consisted of 57% Thy-1⁺, 23% Lyt-1⁺, 6% Lyt-2⁺, and 9% asialo-GM1⁺ cells, while the large cells consisted of 53% Thy-1⁺, 15% Lyt-1⁺, 9% Lyt-2⁺, and 24% asialo-GM1⁺ cells. These findings suggest that IFN-γ-treated Pam 212 cells could stimulate more than one kind of splenic T cell populations. J Invest Dermatol 89:560-566, 1987

I have been demonstrated that keratinocytes [1-3], endothelial cells [4,5], fibroblasts [6], and intestinal epithelial cells [7], which normally lack Ia antigens, begin to express Ia antigens at inflammatory sites. Since these studies, several new lines of evidence suggest that it is interferon-γ (IFN-γ) produced by activated T cells at the inflammatory sites that leads to the induction of Ia antigen expression on a variety of adjacent cell types that do not normally express Ia antigens [8-12].

Ever since Streilein [13] coined the term "skin-associated lymphoid tissues" (SALT) to describe the skin as an immunologic special environment containing Langerhans cells with their antigen-presenting properties and epidermotropic recirculating T-lymphocyte subpopulations (homing T lymphocytes), keratinocytes that may possibly function as immunocompetent cells have also begun to attract interest. This is because keratinocytes have been demonstrated to perform macrophage-like functions, such as endocytosis and phagocytosis [14], production of epidermal thymocyte-activating factor (ETA F) [15,16], and expression of Ia antigens [17,18]. Furthermore, Luger and coworkers [19,20] reported the keratinocyte production of epidermal cell-derived interleukin 3 and epidermal cell-derived natural killer cell-activating factor.

Among various immunologic functions of keratinocytes, the function of Ia antigen-bearing (Ia⁺) keratinocytes has not been clarified yet. Although various kinds of normally Ia⁻ cells express Ia antigens under the stimulation of IFN-γ, their ability to function as antigen-presenting cells is not the same among different cell types. Namely, IFN-γ-treated endothelial cells effectively perform antigen-presenting function, while fibroblasts do not [21]. It is not clear whether IFN-γ-treated keratinocytes carry out antigen-presenting function, although Nickoloff et al [22] recognized that attached human keratinocyte stimulated allogeneic peripheral blood mononuclear leukocytes weakly.

Along with keratinocytes and melanocytes, epithelial cells usually contain Langerhans cells functioning as active accessory cells [23,24], and, in mice, Thy-1 antigen-bearing dendritic cells, whose function is not yet elucidated [25-27]. The presence of these immunocompetent cells makes it difficult to analyze the function of keratinocytes. Pam 212 cells share many characteristics of normal keratinocytes; they have pemphigus antigens, bullous pemphigoid antigens [28], and produce ETA F [15]. Thus, Pam 212 cells become a suitable cell model for analysis of the function of keratinocytes. In this study, we first induced Ia antigen expression on Pam 212 cells by IFN-γ, and then compared DNA synthesis of syngeneic or allogeneic T cells under the stimulation of the Ia⁺ Pam 212 cells.
**Table 1.** [\(^{3}H\)]Thymidine Uptake of C3H T Cells in Mixed Skin Lymphocyte Culture With Pam 212 cells

<table>
<thead>
<tr>
<th>Exp</th>
<th>4 × 10^4</th>
<th>2 × 10^4</th>
<th>1 × 10^4</th>
<th>2 × 10^3</th>
<th>5 × 10^3</th>
<th>2.5 × 10^4</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ-treated Pam 212 cells</td>
<td>8,184 ± 1,815</td>
<td>n.d.</td>
<td>n.d.</td>
<td>57,230 ± 9,030</td>
<td>18,151 ± 1,130</td>
<td>52,071 ± 6,965</td>
</tr>
<tr>
<td>IFN-γ-untrained Pam 212 cells</td>
<td>25,504 ± 1,488</td>
<td>25,437 ± 2,714</td>
<td>41,663 ± 6,508</td>
<td>13,792 ± 922</td>
<td>21,063 ± 1,156</td>
<td>24,432 ± 3,590</td>
</tr>
</tbody>
</table>

*Note: n.d. indicates not determined.*

**MATERIALS AND METHODS**

**Mice** Specific-pathogen-free male mice of BALB/c and C3H/He strain were supplied by the Animal Production Area of Tohoku University School of Medicine (Sendai, Japan). The mice were 8 to 12 weeks old.

**Culture Medium** RPMI 1640 (Grand Island Biological Co., Gibco Ltd., Paisley, Scotland) containing 60 μg/ml kanamycin (Meiji Seika Co. Ltd., Tokyo, Japan) supplemented with 10% heat-inactivated fetal calf serum (Filtron, Pty. Ltd., Altona, Australia), 5 × 10⁻³ M 2-mercaptoethanol, 2 mM glutamine, and 10 mM HEPES (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used for mixed culture of Pam 212 epidermal cells and lymphocytes. RPMI 1640 containing kanamycin, glutamine, and HEPES supplemented with 5% heat-inactivated fetal calf serum was used for culture of Pam 212 cell line.

**Culture of Pam 212 Cell Line** The newborn BALB/c mouse-derived Pam 212 cell line [28] was kindly provided by Dr. Stuart H. Yuspa (Laboratory of Experimental Pathology, National Institutes of Health, Bethesda, MD) and was maintained in monolayer culture in the culture medium mentioned above in a humidified incubator with 5% CO₂ air at 37°C.

To subculture the cells, they were first washed with phosphate-buffered saline (PBS) (pH 7.3) and then treated with 0.25% trypsin in PBS at 37°C for 5 min. After washing the detached cells with the culture medium, their number was counted using a hemocytometer, and the viability was assayed by trypsin blue exclusion.

**Induction of la Antigen on Pam 212 Cells by IFN-γ** Mouse recombinant IFN-γ from *Escherichia coli* was kindly provided by Shionogi Seiyaku Co. Ltd. (Osaka, Japan). We seeded 3 × 10^4 Pam 212 cells on 25-cm² tissue culture flasks (Corning Glass Works, Corning, NY) and cultured in culture medium supplemented with various concentrations of IFN-γ.

**Fluorescence Labeling and Analysis by Fluorescence-Activated Cell Sorter** At various times after culture of Pam 212 cells, the cells were washed to remove nonattached cells and the monolayer was trypsinized for 5 min using 0.25% trypsin and washed with culture medium. Aliquots of 10⁵ viable cells were indirectly stained for 30 min on ice with 150 μl of monoclonal anti-I-A<sup>δ</sup> antibody (IgG2a) (Meiji Institute of Health Science, Tokyo, Japan) at a 1:50 dilution of ascites fluid or control unreactive IgG2a monoclonal antibody. The cells were then washed three times with PBS and stained for 30 min on ice with 150 μl of the F(ab')₂ fraction of FITC-conjugated goat anti-mouse immunoglobulin (Tago Inc., Burlingame, CA) at a 1:30 dilution and washed three times in PBS. After the final wash, the suspension was filtered through gauze to remove any cell aggregates. Ten thousand cells per assay were analyzed by passage through a fluorescence-activated cell sorter (FACS analyzer, Becton-Dickinson). A cytograph was obtained for each sample, with fluorescence intensity (log units) being expressed on the x-axis and cell number on the y-axis.

**Responder T Cell Preparation** Aseptically removed spleens were teased in culture medium and spleen cells were isolated by Ficol-Isoaque centrifugation (the mixture of Ficoll 400 [Phamacia AB, Uppsala, Sweden] and Isopaque 400 [Torii & Co., Ltd., Tokyo, Japan]), diluted with culture medium to a specific gravity of 1.09. T cell-enriched population was obtained after passage through nylon wool column, as described by Julius et al [29]. Nylon-adherent cells were recovered by teasing the nylon wool in cold PBS. Purified T cell populations were obtained by treating T-cell-enriched population with anti-I-A<sup>δ</sup> monoclonal antibody (IgG2a) for BALB/c mice or anti-I-A<sup>δ</sup> monoclonal antibody (IgG2a) (Meiji Institute of Health Science, Tokyo, Japan) (1:50 dilution of ascites fluid) for C3H/He mice for 30 min on ice, followed by 40-min incubation with selected guinea pig complement at 37°C. These procedures yielded more than 95% Thy-1-bearing cells as determined by cytotoxicity test.

**Isolation of T Cell Subsets** Purified Lyt 1⁻ and Lyt 2⁻ cell populations were obtained by treating nylon wool-passed enriched T cell populations with a cocktail of anti-I-A<sup>δ</sup> and anti-Lyt 1.2 (Meiji Institute of Health Science, Tokyo, Japan) or anti-I-A<sup>δ</sup> and Lyt 2.2 antibodies (Meiji Institute of Health Science, Tokyo, Japan) at a 1:50 dilution of ascites fluid, respectively. For 40 min on ice followed by suspension in 1 ml of a 1/10 dilution of guinea pig complement for 45 min. The respective percentage of lysis obtained with each cocktail of monoclonal antibodies plus complement, which was calculated from trypsin blue dye exclusion tests, was approximately 70% for anti-I-A<sup>δ</sup> and anti-Lyt 1.2 and 20% for anti-I-A<sup>δ</sup> and anti-Lyt 2.2 monoclonal antibodies.

**Stimulatory Cells** As stimulator cells, Pam 212 cells or Pam 212 cells cultured with 100 U/ml IFN-γ for 5 days were used after treatment with 60 μg/ml mitomycin-C (Kyouwa Hakko Kogyo Co. Ltd., Tokyo, Japan) for 30 min. Nylon-adherent spleen cells from BALB/C mice were also used after mitomycin-C treatment as a control.

**Mixed Culture of Pam 212 Cells and T-Lymphocytes and Assay of Lymphocyte DNA Synthesis** Synchronous stimulation was assessed by culturing various numbers of Pam 212 cells or IFN-γ-treated Pam 212 cells or nylon-adherent spleen cells from BALB/c mice alone or with 2.5 × 10⁵ BALB/c T cells in 96-well microtiter plates with flat-bottomed wells (No. 3595; Costar, Cambridge, MA) for 5 days. Allogeneic stimulation was assessed by the stimulator cells mentioned above alone or with 2.5 × 10⁵ C3H/He T cells. Four hours before the end of the incubation, 5 μCi of [\(^{3}H\)]thymidine (85 Ci/mmol, Amersham International plc., Buckinghamshire, England) was added to the cultures, and [\(^{3}H\)]thymidine incorporation was measured in a liquid scintillation counter. All the data were expressed as mean cpm ± SD of triplicate cultures. Furthermore, the ratio of maximum stimulation was expressed as maximum [\(^{3}H\)]thymidine uptake by T cells stimulated with IFN-γ-treated Pam 212 cells/maximum [\(^{3}H\)]thymidine uptake by T cells stimulated with IFN-γ-unstimulated Pam 212 cells. [\(^{3}H\)]Thymidine incorporation by C3HHe T cells or by BALB/C T cells was less than 1,000 cpm, when they were cultured alone.

In syngeneic stimulation, purified Lyt 1⁻ or Lyt 2⁻ T cell subset...
of BALB/c mouse spleen cells was used as responder cells, namely 2.5 × 10⁵ Lyt 1⁻ or Lyt 2⁻ cells were cultured with various numbers of Pam 212 cells or IFN-γ-treated Pam 212 cells for 5 days and [³H]thymidine incorporation was assessed as mentioned above. [³H]Thymidine incorporation by Lyt 1⁻ cells or Lyt 2⁻ cells was less than 1,000 cpm, when they were cultured alone.

Blocking of DNA Synthesis of T Cells by Monoclonal Anti-I-A⁺ Antibody The ascites fluid containing monoclonal anti-I-A⁺ antibody (IgG2a) or unreactive IgG2a monoclonal antibody was added to the cultures at a final concentration of 0.2% v/v at the initiation of the culture.

Fluorescence Flow Cytometric Analysis of Proliferating Cells Cultured With IFN-γ-Treated Pam 212 Cells We cultured 2.5 × 10⁶ BALB/c T cells with 2 × 10⁵ IFN-γ-treated Pam 212 cells in 24-well plates (No. 143982; Nunc, Kamstrup, Denmark). After 5 days culture, cytofluorographic analysis of proliferating cells was performed on a FACS analyzer by means of indirect immunofluorescence with fluorescein isothiocyanate (FITC)-conjugated anti-mouse immunoglobulins for anti-Lyt 1.2, anti-Lyt 2.2, and with FITC-conjugated anti-rat immunoglobulins (Tago) for anti-L3T4 and anti-mouse IL 2 receptor (7D4) (a gift from Dr. T. Ito, Department of Anatomy, Tohoku University School of Medicine, Sendai, Japan) antibodies and by means of

Figure 2. The effect of monoclonal anti-I-A⁺ antibody on mixed skin lymphocyte culture of IFN-γ-treated Pam 212 cells and T cells. Monoclonal anti-I-A⁺ antibody (hatched bar) or unreactive isotype monoclonal antibody (empty bar) was added to the culture. The culture was composed of: a, 2 × 10⁵ IFN-γ-treated Pam 212 cells and 2.5 × 10⁵ BALB/c T cells; b, 1 × 10⁵ IFN-γ-treated Pam 212 cells and 2.5 × 10⁵ BALB/c T cells; c, 2 × 10⁴ IFN-γ-treated Pam 212 cells and 2.5 × 10⁵ C3H/He T cells; d, 1 × 10⁴ IFN-γ-treated Pam 212 cells and 2.5 × 10⁵ C3H/He T cells; and e, 2.5 × 10⁵ nylon-adherent BALB/c spleen cells and 2.5 × 10⁵ C3H/He T cells.
Table II. [3H]Thymidine Uptake of BALB/c T Cells* in Mixed Skin Lymphocyte Culture With Pam 212 Cells

<table>
<thead>
<tr>
<th>Exp 1</th>
<th>Exp 2</th>
<th>Exp 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ-treated Pam 212 cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 × 10^6</td>
<td>2,444 ± 861</td>
<td>3,161 ± 861</td>
</tr>
<tr>
<td>2 × 10^6</td>
<td>5,172 ± 923</td>
<td>7,913 ± 1,095</td>
</tr>
<tr>
<td>1 × 10^6</td>
<td>10,741 ± 763</td>
<td>16,617 ± 1,059</td>
</tr>
<tr>
<td>5 × 10^5</td>
<td>10,114 ± 226</td>
<td>16,253 ± 1,117</td>
</tr>
<tr>
<td>2.5 × 10^5</td>
<td>6,733 ± 1,681</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

IFN-γ-untreated Pam 212 cells

| 4 × 10^6 | 594 ± 128 | n.d. | n.d. |
| 2 × 10^6 | 2,418 ± 363 | 4,606 ± 517 | 1,238 ± 168 |
| 1 × 10^6 | 3,031 ± 1,039 | 5,571 ± 486 | 1,771 ± 638 |
| 5 × 10^5 | 3,383 ± 1,913 | 5,398 ± 1,885 | 6,259 ± 1,152 |
| 2.5 × 10^5 | 4,015 ± 2,504 | n.d. | 4,971 ± 3,547 |

Nylon-adherent cells

| 2.5 × 10^5 | 316 ± 192 | 290 ± 82 | 290 ± 82 |

The ratio of maximum stimulation (IFN-γ-treated/untreated)

| IFN-γ-treated/untreated | 2.7 | 1.9 | 4.1 |

*IFN-γ-treated Pam 212 cells were cultured with various numbers of Pam 212 or IFN-γ-treated Pam 212 cells for 5 days and [3H]thymidine incorporation was assessed. BALB/c T cells incorporated less than 1,000 cpm of [3H]thymidine, when cultured alone.

**Pam 212 cells were treated with 100 U/ml recombinant IFN-γ for 5 days (+) or not treated (−).

n.d.: not performed.

RESULTS

Induction of I-A^d Antigen by IFN-γ When Pam 212 cells were treated for 4 days with various concentrations (0, 10, 100, 500, and 1000 U/ml) of IFN-γ, approximately 25% of cells expressed I-A^d antigens at 100 U/ml of IFN-γ or more. Then Pam 212 cells were exposed to 100 U/ml of INF-γ for various time periods (24, 48, 72, 96, and 120 h). It was found that they began to express I-A^d antigens (25%) after 72 h of culture and that the percentage of I-A^d-bearing cells continued to increase thereafter. After 120 h of culture, 66% of cells were found to express I-A^d antigens (Fig 1).

Mixed Cell Culture of IFN-γ-Treated or -Untreated Pam 212 Cells and C3H T Cells Because the IFN-γ-treated Pam 212 cells expressed I-A^d antigens, their ability to stimulate allogeneic T cell DNA synthesis was examined in mixed cell culture consisting of 2.5 × 10^5 C3H T cells/well and various numbers of IFN-γ-treated or -untreated Pam 212 cells (Table I). The IFN-γ-treated Pam 212 cells stimulated allogeneic T cell DNA synthesis. Unexpectedly, however, the IFN-γ-untreated Pam 212 cells also stimulated the allogeneic T cell DNA synthesis. They stimulated the [3H]thymidine uptake of allogeneic T cells to an extent similar to BALB/c nylon-adherent spleen cells. The culture consisting of allogeneic T cells and 1–2 × 10^5 IFN-γ-untreated Pam 212 cells or 5 × 10^5 IFN-γ-untreated Pam 212 cells/well showed maximum [3H]thymidine uptake. The ratio between [3H]thymidine uptake by T cells stimulated with the IFN-γ-treated Pam 212 cells and that by T cells stimulated with the IFN-γ-untreated Pam 212 cells was approximately 3.

Mixed Cell Culture of IFN-γ-Treated or -Untreated Pam 212 Cells and BALB/c T Cells When syngeneic T cells were cultured with the IFN-γ-treated or -untreated Pam 212 cells, again there was stimulation of [3H]thymidine uptake but both cultures (Table II). The maximum response was observed in the culture with 5 × 10^5 × 10^5 Pam 212 cells/well. Regardless of IFN-γ treatment, more prominent [3H]thymidine uptake was observed in the mixed culture of syngeneic T cells and Pam 212 cells than in the mixed culture of BALB/c T cells and BALB/c nylon-adherent spleen cells. The ratio of maximum stimulation comparing between maximum [3H]thymidine uptake by T cells stimulated with IFN-γ-treated Pam 212 cells and that by T cells stimulated with the IFN-γ-untreated Pam 212 cells ranged from 2 to 4.

Blocking of DNA Synthesis of T Cells Stimulated With IFN-γ-Treated or -Untreated Pam 212 Cells by Monoclonal Anti-I-A^d Antibody Monoclonal anti-I-A^d antibody could not inhibit DNA synthesis of C3H T cells stimulated by the IFN-γ-treated or -untreated Pam 212 cells, whereas the same antibody inhibited completely the allogeneic stimulation of C3H T cells by BALB/c nylon adherent spleen cells. Monoclonal anti-I-A^d antibody also could not inhibit DNA synthesis of BALB/c T cells cultured with the IFN-γ-treated or -untreated Pam 212 cells. Since syngeneic mixed lymphocyte culture of BALB/c T cells and BALB/c nylon adherent spleen cells did not induce DNA synthesis, the observed effect of the monoclonal antibody in this culture system was not clear (Fig 2).

Mixed Cell Culture of Pam 212 Cells or IFN-γ-Treated Pam 212 Cells With Purified T Cell Subsets Lyt 1^- cells or Lyt 2^- cells purified from BALB/c spleen cells were cultured with Pam 212 cells or IFN-γ-treated Pam 212 cells, and their [3H]thymidine incorporation was compared with that of unfractonated BALB/c T cells (Table III). Unexpectedly, no difference in [3H]thymidine incorporation was found between Lyt 1^- and Lyt 2^- and T cells, when they were cultured with Pam 212 cells and IFN-γ-treated Pam 212 cells. When stimulated with the IFN-γ-treated Pam 212 cells, both Lyt 1^- and Lyt 2^- cells incorporated almost the same amount of [3H]thymidine as unfractonated T cells.

Surface Phenotype of Proliferating T Cells After 5 Days Culture With IFN-γ-Treated Pam 212 Cells When BALB/c T cells that were cultured with IFN-γ-treated Pam 212 cells for 5 days were viewed under phase-contrast microscope, two types of cells, small lymphoid cells and large round cells, were observed to proliferate on the expanding Pam 212 cells (Fig 3). The cell-size profile using forward scatter by FACS analyzer showed a big peak consisting of small cells that ranged from 50 to 120 in arbitrary unit (a.u.) of forward scatter and a little peak consisting of large cells that ranged from 130 to 220 a.u. (Fig 4). We analyzed the phenotype of small cells ranging from 50 to 120 a.u. and that of large cells ranging from 130 to 220 a.u. Approximately 50% of cells were found to be Thy-1^- in both cell populations; the percentage of Lyt-1^- cells in the small cell population was more than that in the large cell population, while that of the asialo-GM1^- cells is less in the former population than that in the latter (Table IV).

DISCUSSION

Pam 212 cells share many characteristics with normal keratinocytes [17,28], and in this study we demonstrated that they could express Ia antigens under the stimulation of IFN-γ as normal keratinocytes. Ia^+ Pam 212 cells began to appear 72 h after exposure to IFN-γ and increased in number, comprising approximately 60% of cells at 120 h. They do not contain Ia^+ Langerhans cells (M. Iijima et al, unpublished observations), although normal epidermal cells from mice usually contain Ia^+ Langerhans cells
Table III. [3H]Thymidine Incorporation of BALB/c T Cell Subsets Stimulated by Pam 212 Cells or IFN-γ-Treated Pam 212 Cells

<table>
<thead>
<tr>
<th>T cell subsets</th>
<th>IFN-γ</th>
<th>Numbers of Pam 212 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 x 10⁴</td>
</tr>
<tr>
<td>Ia⁺ cells</td>
<td>+</td>
<td>5,483 ± 1,478</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>1,317 ± 206</td>
</tr>
<tr>
<td>Lyt 1⁻ (Lyt 1⁻ Ia⁺)</td>
<td>+</td>
<td>6,001 ± 2,006</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>2,706 ± 834</td>
</tr>
<tr>
<td>Lyt 2⁻ (Lyt 2⁻ Ia⁻)</td>
<td>+</td>
<td>6,381 ± 1,613</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>1,730 ± 241</td>
</tr>
</tbody>
</table>

*2.5 x 10⁵ BALB/c T cells (Ia⁻), Lyt 1⁻ or Lyt 2⁻ cells were cultured with various numbers of Pam 212 or IFN-γ-treated Pam 212 cells for 5 days and [3H]thymidine incorporation was assessed. Incorporation of [3H]thymidine by all T cell subsets was less than 1,000 cpm, when cultured alone.

Figure 3. Phase-contrast microscopic view of proliferating cells. BALB/c T cells were cultured with IFN-γ-treated Pam 212 cells for 5 days. Small lymphoid cells and large round cells were proliferating on expanded Pam 212 cells.

and Thy-1 antigen-bearing dendritic cells. Therefore, Ia⁺ Pam 212 cells seem to present a suitable cell model for analysis of the function of Ia⁺ keratinocytes.

Ia antigens are reported to be induced in many kinds of cells by IFN-γ. However, the function of Ia antigen induced on normally Ia⁻ cells is not always clear. Nickoloff et al [22] demonstrated that Ia antigen-bearing human keratinocytes stimulated DNA synthesis of allogeneic peripheral blood mononuclear cells weakly. However, it is not clear from their data whether this stimulation is related to antigen-presenting function of Ia⁺ keratinocytes, because inhibition of this allogeneic stimulation by anti-Ia antibody was not performed. In contrast, Czernielerwski [30] and Yoneda et al [31] failed to show any active proliferation of peripheral blood leukocytes with the stimulation by Ia⁺ keratinocytes as compared with that by Ia⁻ keratinocytes. Our present study demonstrated that Ia⁺ Pam 212 cells stimulated DNA synthesis of both syngeneic T cells and allogeneic T cells much more effectively than Ia⁻ Pam 212 cells. We think that this discordance with the findings of other workers results from the difference of the experimental system; we used the mouse-purified splenic T cells as responding cells, while the authors mentioned above used human peripheral blood leukocytes as the responding cells. Furthermore, we found that the stimulation of DNA synthesis in these T cells was unable to be inhibited by monoclonal anti-I-A¹ antibody. This makes a sharp contrast to the DNA synthesis of T cells stimulated with allogeneic nylon-adherent cells that was completely inhibited by the same treatment. These findings suggest that the stimulation of syngeneic and allogeneic T cells by Ia⁺ Pam 212 cells is unlikely to be due to the antigen-presenting function of Pam 212 cells, although we can not still negate the possibility that Pam 212 cells may function as antigen-presenting cells through I-E antigens.

Pam 212 cells are reported to produce epidermal cell-derived interleukin 3 [19] and keratinocyte T cell growth factor (KTGF) [32]. Interleukin 3 induces continuous proliferation of Thy-1⁺, Lyt 1⁻ spleenic cells in BALB/c mice [33] and KTGF also stimulates only IL 2 dependent T cell line with helper T phenotype [32]. The stimulation of resting T cells with Pam 212 cells without IFN-γ treatment as observed in our study is suspected to be attributable at least partly to the production of these factors from Pam 212 cells. Furthermore, the finding that IFN-γ-treated Pam 212 cells stimulated DNA synthesis of T cells more effectively than those untreated by IFN-γ might be ascribed to more vigorous production of them by the former than the latter. In fact, recently we found with four-fold concentrated culture supernatant of IFN-γ-treated Pam 212 cells that it stimulated syngeneic T cells maximally at the concentration of 12.5% (to be published in full elsewhere).

Using purified T cell subsets, we found that both Lyt 1⁻ and Lyt 2⁻ cells could incorporate [3H]thymidine in amounts similar to that of unfractonated T cells with the stimulation of Pam 212 cells, especially with that of IFN-γ-treated cells. The proliferating cells after 5 days culture with IFN-γ-treated Pam 212 cells were composed of two morphologically different cell populations, including a large number of Lyt 1⁻ and Lyt 2⁻ cells. This finding means that most of the [3H]thymidine incorporation of Ia⁻ T, Lyt 1⁻, or Lyt 2⁻ cells in our study might result from the proliferation of these Lyt 1⁻ and Lyt 2⁻ cells. It is also suggested that some factors other than epidermal cell-derived interleukin 3...
of infiltrating lymphocytes in the skin, especially those in the epidermis, much more efficiently than la keratinocytes.

REFERENCES


3. Aiba S, Tagami H: HLA-DR antigen expression on the keratinocyte surface in dermatoses characterized by lymphocytic exocytosis (e.g. pityriasis rosea). Br J Dermatol 111:285-294, 1984


