Characterization of Distribution of T Lymphocyte Subsets and Activated T Lymphocytes Infiltrating into Sarcoid Lesions

Shinji Kita, Tomiyasu Tsuda, Katsunori Sugisaki, Eishi Miyazaki and Tetsuro Matsumoto

We studied the relationship between various T lymphocyte functions and granuloma formation in 5 lung tissue and 4 lymph node tissue samples from patients with sarcoidosis by immunohistochemical methods. In the lesion of sarcoidosis, T cells were positive for αβ TCR, but γδ TCR-positive T cells were rarely observed. The results of analysis of functional subsets showed that T cells in the internal area of granuloma were predominantly helper/inducer subset (CD4+CD45RA+). On the other hand, cytotoxic T cells (CD8+CD45RA+CD11b+) were present in abundance in the outer boundaries of granuloma. In addition, suppressor-inducer T cells (CD4+CD45RA+) were present in the surrounding areas. However, T cells of various subsets were present sporadically outside the granulomas. We also studied the distribution of T cells expressing activation-related antigens. The results showed that T lymphocytes in the internal area of granulomas more frequently had these antigens than did T lymphocytes in the external area. These findings suggested that T cells infiltrating into the sarcoidosis lesion demonstrated a layer-like distribution based on functional subsets. These findings also confirm that activated T cells were more abundantly distributed in the internal area of sarcoid granuloma than in the external area.

Key words: sarcoidosis, histochemistry, immunofluorescence double staining, T cell subsets, activated T cell

Introduction

Sarcoidosis is a systemic disease in which epithelioid cell granuloma of unknown origin occur in organs throughout the body. To clarify the pathogenesis of this disease, various studies have been designed covering not only sarcoidosis but also including other granulomatous diseases which exhibit a similar pathogenesis as a disorder manifesting delayed-type hypersensitivity such as tuberculosis and mycosis infection (1, 2). Recently these granulomatous diseases have been characterized on the basis of pathological and immunological findings. This research investigates the relationship between T lymphocyte function and sarcoid granuloma formation using immunohistochemical techniques.

Recently, it has been reported that T lymphocytes, which promote granulomas, have an αβ type T cell receptor (TCR) and not a γδ type TCR (3). Other reports state that CD4+ T cells are distributed diffusely throughout the granuloma lesions while the CD8+ T cells are found in only the outer part of granulomas (4, 5).

Previously, we reported on the distribution of T lymphocytes with the leukocyte common antigen (LCA) isoform (6). In that study, we demonstrated that in granulomatous lesions CD45RO+ T cells (memory T) were in abundance in the internal and external areas, and CD45RA+ T cells (naive T) were in the external area only. Recent studies have distinguished T lymphocytes subsets by using the combination of LCA isoforms and lymphocyte surface markers (CD4 and CD8) in vitro (7, 8). Here, we attempted to clarify the functional subsets of T lymphocytes using similar concepts. We distinguished the cell type of T lymphocytes in sarcoidosis lesions by cell type: helper/inducer T (CD4+CD45RA+); suppressor-inducer T (CD4+CD45RA+); suppressor T (CD8+); cytotoxic T (CD8+CD45RA+CD11b+) (9), using the double staining method with anti-CD4, CD8, CD45RA and CD11b antibodies. From our previous findings as mentioned above, there was almost no naive T cell infiltration into the internal area of granuloma lesions unless there was memory T cell infiltration. We divided the granulomatous lesions into internal and external areas. In this study we stained the T cell activation-related antigens such as CD11a, CD25, CD26, CD28, CD29, CD43, CD54, CD58 and CD69 using monoclonal antibodies. We counted positive...
cells for each antigen in the granuloma lesion to clarify the immunological differences in each area.

Materials and Methods

Materials
We used lung tissues obtained from 5 patients with sarcoidosis by open lung biopsy via a thoracotomy between 1986 and 1990, and the lymph nodes obtained from 4 patients (three cervical lymph nodes and one mediastinal lymph node).

Immunohistochemistry
Preparation of frozen sections
We placed each sample obtained from the subjects into a receptacle (1 cm in diameter, 2 cm deep) made of aluminum foil, and then covered it with TISSUE-TEK® (Miles Inc, Elkhart, IN). The sample was placed in a beaker with acetone dry ice to freeze, and then cut into 4 μm sections by a cryostat. The frozen section was placed on a gelatin-coated slide and immediately dry-fixed it with cool jet air and then fixed with acetone for 8 minutes at 4°C. Next, the slide was washed with phosphate-buffered saline (0.01 M, pH 7.2, PBS) for 10 minutes at room temperature. The circumference of tissue on the slide was wiped well and prepared for staining.

Monoclonal antibodies
We used anti human mouse monoclonal antibodies as shown in Table 1. All immunoglobulin isotypes were of the IgG class.

Table 1. Monoclonal Antibodies Used in This Study

<table>
<thead>
<tr>
<th>Monoclonal antibodies</th>
<th>Source</th>
<th>Immunoglobulin isotype</th>
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<tbody>
<tr>
<td>TCRα (αF1)</td>
<td>T cell sciences</td>
<td>IgG2a</td>
</tr>
<tr>
<td>TCRβ (βF1)</td>
<td>T cell sciences</td>
<td>IgG1</td>
</tr>
<tr>
<td>TCRγ (CyM1)</td>
<td>T cell sciences</td>
<td>IgG1</td>
</tr>
<tr>
<td>TCRδ (δTCS1)</td>
<td>T cell sciences</td>
<td>IgG1</td>
</tr>
<tr>
<td>CD4 (OKT4)</td>
<td>Ortho diagnostic system</td>
<td>IgG2b</td>
</tr>
<tr>
<td>CD8 (OKT8)</td>
<td>Ortho diagnostic system</td>
<td>IgG2a</td>
</tr>
<tr>
<td>CD4-PE conjugated</td>
<td>DAKO</td>
<td>IgG1</td>
</tr>
<tr>
<td>CD8-PE conjugated</td>
<td>Coulter immunology</td>
<td>IgG1</td>
</tr>
<tr>
<td>CD11a</td>
<td>DAKO</td>
<td>IgG1</td>
</tr>
<tr>
<td>(anti human LFA-1 alpha chain)</td>
<td>Ortho diagnostic system</td>
<td>IgG2b</td>
</tr>
<tr>
<td>CD25 (anti IL-2R)</td>
<td>Becton-Dickinson</td>
<td>IgG1</td>
</tr>
<tr>
<td>CD26</td>
<td>Cosmo-Bio</td>
<td>IgG2a</td>
</tr>
<tr>
<td>CD28 (KOLT-2)</td>
<td>Nitirei</td>
<td>IgG1</td>
</tr>
<tr>
<td>CD29 (4B4)</td>
<td>Coulter immunology</td>
<td>IgG1</td>
</tr>
<tr>
<td>CD43 (Anti Leu22)</td>
<td>Becton-Dickinson</td>
<td>IgG1</td>
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<tr>
<td>CD45RA (2H4)</td>
<td>Coulter Clone</td>
<td>IgG1</td>
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<tr>
<td>CD45RO (UCHL-1)</td>
<td>DAKO</td>
<td>IgG2a</td>
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<td>CD54 (Anti human Leu 54)</td>
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<tr>
<td>CD58 (Anti human LFA3)</td>
<td>Cosmo-Bio</td>
<td>IgG2a</td>
</tr>
<tr>
<td>CD69</td>
<td>Cosmo-Bio</td>
<td>IgG2b</td>
</tr>
</tbody>
</table>

Single staining
PAP-Kits (Cambridge Research Laboratory, Cambridge, MA, or DAKOPATTS, Carpinteria, CA) were used. As a blocking solution, a mixture of goat and rabbit serum in equal volumes diluted ten times with physiological saline was used.

Double staining
Double staining was performed according to the modified method of Kupper and Storz (10). Anti-CD45RA or anti-CD11b monoclonal antibodies were used as the primary antibody. After one hour incubation, the slide was washed three times with PBS for five minutes. Then, the sample was incubated for one hour with biotinylated anti-mouse IgG antibody and washed the slide with PBS, and then incubated for one hour using fluorescein isothiocyanate (FITC)-labeled avidin. Then the slide was washed again with PBS, and blocked the sample with 5% normal mouse serum. The sample was incubated for one hour with phycoerythrin (PE)-labeled second monoclonal antibody (PE-conjugated CD4 or CD8), followed by a final washing with PBS. All incubation steps for double staining were carried out in a moist chamber at 4°C. Then, the slide was embedded and photographed using a fluorescence microscope (Axophot, Carl Zeiss). Finally, the cells were judged for staining.

Evaluation of double staining
It is ideal that we employ the combination anti-CD4 antibody with anti-CD45RA or anti-CD45RO antibodies, or the combination of anti-CD8 antibody with anti-CD45RA or anti-CD45RO antibodies. In this study, however, we did not use anti-CD45RO antibody, because our previous single staining with anti-CD4 and anti-CD45RO antibodies produced staining of epithelioid cells as well as T lymphocytes. In principle, the peripheral blood T cells can be divided into two types at the peripheral blood levels: CD4+ and CD8+, or CD45RA+ and CD45RO+. Thus, we performed staining using a combination PE-conjugated anti-CD4 antibody and FITC-conjugated anti-CD45RA antibody, and also by a combination of PE-conjugated anti-CD8 antibody with FITC-conjugated anti-CD45RA antibody. We evaluated the results of the double staining as follows: cells stained red were judged to be CD4+CD45RA− or CD45RO−, or CD8+CD45RA+ or CD45RO+. We performed staining using a combination PE-conjugated anti-CD4 antibody and FITC-conjugated anti-CD45RA antibody, and also by a combination of PE-conjugated anti-CD8 antibody with FITC-conjugated anti-CD45RA antibody. We evaluated the results of the double staining as follows: cells stained green were treated as CD4~CD45RA− or CD8~CD45RA+ T cells; cells stained yellow were judged to be CD4+CD45RA− or CD8+CD45RA+ T cells; cells stained green were treated as CD4+CD45RA+ cells or CD8+CD45RA+ cells. In addition, we performed double staining using PE-conjugated anti-CD8 antibody and FITC conjugated anti-CD11b antibody.

Evaluation of granulomatous lesions on the basis of site affected
The lesion of sarcoidosis can be divided into two parts the inner site of epithelioid cell aggregation and outer site of infiltration by the cells, mainly lymphocytes. Some reports indicate that CD4+ cells are found diffusely in large quantities in both the inner aggregate of epithelioid cells and outer part, while CD8+ cells mainly infiltrate the boundary of the aggregate of epithelioid cells (4, 5). Utilizing these findings, we divided...
T Cells Infiltrating into Sarcoid Lesion

Figure 1. Division of the granulomatous lesion. The lesions of the sarcoidosis are divided into the two compartments, aggregate site of epithelioid cells (internal area) and the site outside of the aggregation of epithelioid cells (external area). Moreover, the external area is divided into the two regions; outer boundary zone and outer zone.

the granulomatous lesion of sarcoidosis into three areas in order to evaluate the distribution of T cell subsets (Fig. 1).

(A) internal area; aggregate of epithelioid cell
(B) external area
   (a) outer boundary zone
   (b) outer zone

Calculation of antigen-positive cells

We detected antigen positive T cells a graphic analyzing system (Nikon Cosmozone IS, Nikon, Tokyo), and detected antigen-positive lymphocytes macroscopically (we did not use an automatic analyzer) with the slide of the graphic image of the single staining sample projected on the color display. T lymphocytes usually could be recognized as ring-like staining cells. The number of positive cells per square millimeter (positive cell count) of 10–20 different areas in the internal and the external areas of each granulomatous lesion were counted. We added the cell counts of CD4+ T lymphocytes and that of CD8+ T lymphocytes from the serial sections to determine the total T lymphocyte count of each sample. The percentage of antigen-positive T cells were counted according to the following equation.

We counted the cells in the internal and external areas in the lesion of lung by a paired t-test using the calculated percentage as below.

Percentage of lymphocytes stained with a monoclonal antibody =

\[
\frac{\text{Number of T cells stained with a monoclonal antibody per mm}^2}{\text{Number of CD4}^+ \text{ cells per mm}^2 + \text{Number of CD8}^+ \text{ T cells per mm}^2}\times 100
\]

However, in the lymph node granuloma lesions, we were not able to make any measurements because of the difficulty in discriminating the infiltrating cells from the existing T cells.

Figure 2. TCR expression by T cells in the lung tissues from a patient with sarcoidosis. T cells expressing αβTCR are identified with the monoclonal antibodies, αF1 (a) and βF1 (b). T cells expressing γδTCR are identified with the monoclonal antibody, δTCS1 (c). The majority of the T cells in the internal and external areas of the granuloma express the αβ TCR. The γδ TCR positive T cells are rare (Calibration bars = 100 μm).
Results

TCR

In all of the tissue samples from sarcoidosis granuloma of lung and lymph node, the T cells showed αβ type TCR (Fig. 2a and 2b). T cells with γδ type TCR were infrequently seen in the lesions (Fig. 2c).

CD4+ and CD8+ T cells

We studied five lung tissues with sarcoidosis and four lymph node tissues with sarcoidosis, the ratio of CD4 to CD8 was 2.8 in internal area and 1.9 in external area. This difference was significant as p<0.05. In the lymph nodes, the ratio of CD4 to CD8 was 2.5 in the internal area.

Disposition of T lymphocyte subsets in each area of the granuloma

As in Fig. 3a, a single staining showed the diffuse distribution of CD4+ T cells in both internal and external areas. Conversely, we found abundant CD8+ T cells in the outer boundary zone, while we found only in small numbers of these cells in the internal area (Fig. 3b). CD45RO+ T cells were found in both internal and external areas, but CD45RA+ T cells were infrequently seen in the internal area (Fig. 3c and 3d).

Subsequently, we examined the T cell subsets by double staining. Figure 4 shows the results of this procedure: An examination of T cell subsets using a combination of anti-CD4 and anti-CD45RA monoclonal antibodies demonstrated that only CD4+CD45RA− T cells were found in the internal area, while CD4+CD45RA+ T cells were in the outer zone especially near the outer boundary zone. Also, there was a scattered distribution of CD4+CD45RA− T cells and CD4+CD45RA+ T cells in the outer zone. Conversely, as shown in Fig. 5, an examination of the T cell subsets using a combination of anti-CD8 and anti-CD45RA monoclonal antibodies demonstrated the distribution of CD8+CD45RA− T cells in the outer boundary zone, while in the internal area, we could find only small numbers of these cells. CD8+CD45RA+ T cells were found in the outer zone. We performed double staining using a combination of anti-CD8 and anti-CD11b monoclonal antibodies, however, no CD8+CD11b+ T cells could be found throughout the granulomatous tissues (Fig. 6). These distributions of T

Figure 3. Single immunostaining of a frozen section of lung tissue from a patient with sarcoidosis. CD4+ T cells are distributed both in the internal and the external areas of the granuloma (a). CD8+ T cells are distributed mainly in the outer boundary zone (b). CD45RO+ T cells are found both in the internal and the external areas (c). CD45RA+ cells are not seen in the internal area (d). (Calibration bars = 100 μm)
Figure 4. Immunofluorescence double staining of frozen section from a patient with sarcoidosis using a combination of anti CD4-PE and anti CD45RA-FITC. In the internal area of the granuloma, the majority of T cells are CD4⁺CD45RA⁻ cells (red fluorescence). Both CD4⁺CD45RA⁻ T cells and CD4⁺CD45RA⁺ T cells (yellow fluorescence) are seen in the external area. CD4⁺CD45RA⁺ T cells are distributed in the outer zone particularly in proximity to the outer boundary zone. Green fluorescent cells are defined as CD4⁻CD45RA⁺ T cells or CD45RA⁺ B cells. These findings in the section of the lung (a) resemble the findings in the lymph node (b). (Calibration bars = 100 μm)

Figure 5. Immunofluorescence double staining of frozen section from a patient with sarcoidosis using a combination of anti CD8-PE and anti CD45RA-FITC. CD8⁺CD45RA⁻ T cells are distributed in the outer boundary zone (red fluorescence). CD8⁺CD45RA⁺ T cells are distributed in the external area only (yellow fluorescence). Green fluorescent cells are defined as CD8⁻CD45RA⁺ T cells or CD45RA⁺ B cells. In the internal area of granuloma, CD8⁺ T cells are infrequently seen. These findings in the section of the lung (a) resemble the findings in the lymph node (b). (Calibration bars = 100 μm)

Figure 6. Immunofluorescence double staining of a frozen section of a lymph node from a patient with sarcoidosis using a combination of anti CD8-PE and anti CD11b-FIRC. CD8⁺CD11b⁻ T cells are distributed in external area (red fluorescence). CD8⁺CD11b⁺ T cells are not seen in both the internal and the external areas. (Calibration bars = 100 μm)
Sarcoidosis is a disease responsible for the emergence of epithelioid noncaseating granulomas occurring throughout the body. The cause of this disease is not yet known, however sarcoidosis has pathological findings closely resembling those of infectious granuloma such as tuberculosis. In the analysis of T lymphocyte subsets, van den Oord et al reported, no differences between the distribution of subsets in both sarcoidosis and tuberculosis affected tissues (5). It would not be incorrect to say that sarcoidosis is a disease, like tuberculosis, based on delayed-type hypersensitivity. We studied T lymphocyte subsets by looking at T cell receptor antigens before analyzing the subsets. A recent report revealed that γδ TCR-positive T lymphocytes at peripheral blood increased in granulomatous diseases such as sarcoidosis, and suggest an increase at the tissue level (11). However, only one to two T lymphocytes of this type were seen in the entire visual field in the lung and lymph node lesions throughout the study. Furthermore, practically all T lymphocytes observed were γδ TCR-positive. Therefore, it became clear that the T lymphocytes involved in the formation of epithelioid cell granulomas are γδ TCR-positive T lymphocytes. Our results agreed with the study of Tazi et al (3).

Next, we attempted to analyze the functional subsets of T lymphocytes. Functional subsets of the T lymphocytes reported so far are helper cells, helper inducer cells, cytotoxic cells, suppressor cells, suppressor inducer cells, and killer cells. However, these functional subsets were originally designated as such based on their tumor immunity and their effects on the ability of B lymphocytes to produce antibodies. It appears that the mechanism by which these subsets act directly on delayed-type hypersensitivity has not been fully substantiated. We therefore undertook the present study by i) analyzing the performance of the functional subsets of the T lymphocyte in lesion of delayed-type hypersensitivity, in terms of their distribution pattern using sarcoidosis lesions, and thereby ii) attempting to assess whether the functional subsets are appropriately termed so as to fit the pathology of lesions of delayed-type hypersensitivity.

After initiating the present study, we were faced with the problem of how to define T lymphocyte subsets. We resolved to take the following steps: First, we decided on the following subsets by using a combination of CD8 antigen with CD1 lb antigen. Recently, helper/inducer cells (CD4+CD1 lb), cytotoxic cells (CD8+CD45RA~ (CD45RO+)), suppressor-inducer cells (CD4+CD45RA~ (CD45RO+)), cytotoxic cells (CD8+CD45RA~ (CD45RO+)), suppressor cells, suppressor inducer cells, and killer cells. These functional subsets were originally designated as such based on their tumor immunity and their effects on the ability of B lymphocytes to produce antibodies. It appears that the mechanism by which these subsets act directly on delayed-type hypersensitivity has not been fully substantiated. We therefore undertook the present study by i) analyzing the performance of the functional subsets of the T lymphocyte in lesion of delayed-type hypersensitivity, in terms of their distribution pattern using sarcoidosis lesions, and thereby ii) attempting to assess whether the functional subsets are appropriately termed so as to fit the pathology of lesions of delayed-type hypersensitivity.

After initiating the present study, we were faced with the problem of how to define T lymphocyte subsets. We resolved to take the following steps: First, we decided on the following subsets by using a combination of the LCA isoform with CD4 and CD8 antigens, which is a combination often reported on recently; helper/inducer cells (CD4+CD45RA~ (CD45RO+)), suppressor-inducer cells (CD4+CD45RA~ (CD45RO+)), cytotoxic cells (CD8+CD45RA~ (CD45RO+)), suppressor cells, suppressor inducer cells, and killer cells. Then we identified the killer cells (CD8+CD1 lb) and cytotoxic cells (CD8+CD1 lb) by using a combination of CD8 antigen with CD1 lb antigen. The present study clarified the following points: 1) Single staining shows the diffuse presence of CD4+ T cells and CD45RA+ cells in both the internal and external areas of the granuloma; 2) CD8+ cells are practically absent in the internal area of the granuloma; and 3) CD45RA+ cells are also practically absent in the internal area of the granuloma. These results prompted us to perform double staining, and thereby to determine the functional subsets of T lymphocytes. As shown in Figs. 4, 5 and 6, assessment by double staining showed that practically only memory type helper/inducer cells of CD4+CD45RO+
CD45RA- infiltrated the internal area of the epithelioid cell granuloma, with cytotoxic cells of CD8*CD45RO+ (CD45RA-CD11b-) in the outer boundary zone of the granuloma, surrounding the former. Double staining also disclosed a scheme in which suppressor-inducer cells of the CD4*CD45RA+ cells are distributed in the outer zone, outwards from the cytotoxic cells mentioned above. Moreover, we saw various cells sporadically infiltrating in the external area with abundant infiltration of CD8+ T cells as compared to internal area. In internal area of the granuloma, only memory or effector cells exist, as Semenzato et al reported (12), particularly memory cells of CD4+. This fact suggests the existence of some persistent etiologic antigen(s) at this site, as reported by du Bois et al (13). Furthermore, it probably indicates the importance of the existence of CD4+ memory T cells for the formation, maturation, and maintenance of the epithelioid cells granuloma. On the other hand, the granuloma is encircled by cytotoxic T cells of CD8*CD45RO*CD11b-, located in the outer boundary zone. We observed abundant suppressor-inducer cells of CD4*CD45RA+ in the outer zone particularly near the outer boundary zone. Their presence suggests that this region is an aggregate of cells common immunological function directed at restraining the growth of the granuloma, although this region is probably of less significance. Some reports in recent years have argued that the memory T cell (CD45RO+ T) and naive T cell (CD45RA+ T) play different functional roles. The memory T cell is thought to react efficiently to a recall antigen and secretes IL-2, IL-4, IL-3, IL-6, and γIFN in large quantities (14). Meanwhile, it has been reported that the naive T cell reacts effectively to the primary antigens and secretes IL-2 in greater quantities than the memory T cell (14). It has also been reported that the naive T cell differentiates and matures into the memory T cell on the stimulation with PHA and IL-2 (15), and that the presence of IL-6 and macrophages are important in the process of its differentiation and maturation into the memory T cell (16). Judging from these reports and the results of our present study, such distribution of the tissue indicate that the names of the T cell-subsets are of great immunological significance in the process of delayed-type hypersensitivity.

Then, because activated T lymphocytes, especially the memory T lymphocyte, were occupying the interior of granulomas, we studied membranous antigens such as CD11a, CD25, CD26, CD28, CD29, CD43, CD54, CD58, and CD69, which are believed to manifest themselves by being involved in the activation of T lymphocytes. It is evident today that CD11a and CD18 exhibit α and β chains of leukocyte function associated antigen-1 (LFA-1), with their ligands being intercellular adhesion molecule-1 (ICAM-1) (17) and ICAM-2 (18). The relationship between these receptors and ligands resembles the CD2-LFA-3 relationship in that the emergence of a signal between the TCR-CD3 complex and the MHC+ specific antigen causes changes in the structure of LFA-1, and thereby markedly strengthens avidity to attach to ICAM-1 (19). The CD54 is known to be ICAM-1. This antigen was suggested to work for the presentation of antigens to the T cells and macrophages (20). It is also known that the manifestation of the CD54 antigen is induced by inflammatory cytokines (21, 22). CD25 is expressed the activated T cell (23). From the inability of the memory T cell to proliferate when treated with anti-CD25, it is equally obvious that the memory T cell divides and proliferates by the mediation of CD25 (24). Fox et al reported that the CD26 antigen was strongly expressed on activated T cells, suggesting that CD26 antigen is a T cell-activating antigen (25). Moreover, the CD26 antigen contains dipeptidyl peptidase IV, which is said to be a protein that binds to collagen in the rat hepatocyte system (26). This suggests involvement of the CD26 antigen in the activation of the collagen-induced T cell. Furthermore, modulation of CD26 with 1F7, a monoclonal antibody to CD26, potentiates the activation by CD26 as well as the CD3 and CD2 pathways, accompanied by an increase in the intracellular level of Ca2+ (27). It is also known that CD26 associates with the CD45RO (CD45=180kD isoform) (28). Then, it became clear that CD28 operates as a receptor for the B7/BB-1 antigen (29) that has been reported as a B cell activating antigen. Thus, CD28 works as a receptor for the adhesion of cells (30). It has been suggested that the CD28 antibody can amplify the activation by primary signals such as anti-CD3 antibodies and PHA (31). The CD29 antigen was formerly compared to CD45RO since it exhibits the same state in the manifestation of CD45RO in the T lymphocyte. Recently, however, CD29 is thought to be an antigen showing αβ chain of very late antigen (VLA) and therefore to be a receptor to the extracellular matrix (ECM) (32, 33). The activation of the T cell occurs with adhesion to these. CD43 is a 115 kD O-linked glycoprotein (34) called sialophorin or leukosialin. Activation of lymphocytes occurs with the action of L10 (antibody to the CD43) alone, followed by activation of protein kinase C, triggering the increase in Ca2+ in the cell (35). These findings further support that the CD43 antigen couples the phospholipase C/phosphoinositide signal pathways. Recently, ICAM-1 has been presented as a ligand for CD43 (36). The CD69 antigen is called the activation inducer molecule (AIM) (37), and is a phosphorylated disulphide-linked homodimer (38). Its manifestation in the activated T cell occurs prior to the appearance of IL-2R (39). In the present study of the membranous antigens that are involved in the activation of T lymphocytes, we focused on those antigens we have described in this paper, and counted the cells possessing each antigen in the internal and external areas of the granuloma. The results of the present study show that the number of T lymphocytes possessing these antigens is exceedingly large in the internal area. We could not adopt CD3+ cells as pan-T cells, because T cells in the internal area of granuloma were stained weakly compare with these in the external area (data not shown). Kan et al reported that CD3 antigen disappears from the membrane of T cells activated with anti CD3 antibody (40). As is clear from the fact that the internal area of granuloma is occupied by CD45RO+ T lymphocytes alone, the internal area is immunologically very unique. It is understandable that T lymphocytes present in internal area maintain the manifestation of numerous membranous antigens that are related to the activation of T lymphocytes. Furthermore,
the occurrence of such an activation in the internal area would suggest that there is persistent presentation of some causative antigens by the epithelioid cells therein.

In the present study, the percentage of positive cells were calculated on more than 100% in several antigens. This may be attributable to the following conditions: 1) B lymphocytes have antigens common to T lymphocytes; 2) the presence of CD4^+CD8^+ double positive T cells; 3) errors in computation because the tissue used was not identical though the sections used were serial. Under the actual conditions, we think 1) or 3) are the most likely causes. Our previous studies show that epithelioid cells in granulomas manifest numerous monokines and cytokines including IL-2, IL-6, αTNF, αIFN, α or β IL-1 (data unpublished; these monokines and cytokines were excluded from the present study). This result suggests a strong mutual amplifying action on the activation of T cells and epithelioid cells. This strong amplifying action on the activation suggests part of the "raison d’etre" of granuloma in granulomatous inflammation.

As described in this paper, we analyzed the pathology of granulomatous lesions, focusing on sarcoidosis, using immuno-histochemical techniques. Our analysis disclosed a layered distribution of functionally differentiated T lymphocytes of various types inside and outside the granuloma, and clarified that T cells in the internal area are involved in granuloma formation, while the outer boundary zone consisting of cells surrounding the internal area, fulfills the function of suppressing granulomatous formation. Moreover, T lymphocytes in the internal area were found to manifest the activation-related antigens of various types, strongly indicating that the internal area is an aggregate of highly activated cells. Further studies are needed to elucidate the causative antigens as well as the functionally differentiated T lymphocytes in terms of their overall actions exerted through the mediation of cytokines.

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References

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