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CLINICAL REPORT

Activated and Mature CD83-positive Dendritic Cells and Interferon-γ-positive Cells in Skin Eruptions of Secondary Syphilis

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Dendritic cells are considered to be the most potent antigen-presenting cells, and CD83 is expressed at a high level in immunocompetent, activated and mature dendritic cells. Various pathogens can activate and modulate the function of dendritic cells. The presence of activated and mature dendritic cells in skin lesions of secondary syphilis has never been reported. In the present study, an immunohistochemical technique was used to determine the exact tissue distributions of CD83$^+$ dendritic cells and interferon-γ$^+$ cells in skin lesions of patients with secondary syphilis. Immunohistochemical staining was performed by using formalin-fixed, paraffin-embedded sections. A small but significant subpopulation of CD83$^+$ dendritic cells was found in the dermis. CD83$^+$ dendritic cells were in close contact with lymphocytes. High-intensity staining of CD83 antigens was detected not only on the surface but also in the cytoplasm of dendritic cells. Infiltrating mononuclear cells were stained positively for CD4 or CD8, with CD8$^+$ cells always being in the majority. A small number of interferon-γ$^+$ cells resembling mononuclear lymphoid cells were detected in all samples. These results provide *in vivo* support for the hypothesis that dendritic cells are activated by *Treponema pallidum* and that thus activated and mature CD83$^+$ dendritic cells may play a role in the Th1 response in secondary syphilis. *Key words: CD83; dendritic cell; interferon-γ; syphilis.*

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Syphilis is a sexually transmitted disease caused by the spirochetal bacterium *Treponema pallidum*. Basic immune mechanisms that might lead to protective immunity against *T. pallidum* in humans are largely unknown. Cell-mediated immune processes are thought to play a prominent role in the clinical manifestations of syphilis (1 – 4).

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) and are believed to be crucial for the initiation of T-cell response (5). Activated and mature DCs are characterized by the expression of CD83, a member of the immunoglobulin superfamily (6). This process, activation or maturation, is thought to be triggered *in vivo* by exposure to various microbial agents (7). There is now evidence from both *in vivo* and *in vitro* studies to support the notion that *T. pallidum*'s membrane lipoproteins efficiently activate DCs (8, 9). Thus changes in the number or localization of activated and mature CD83$^+$ DCs could be expected *in situ* in the skin lesions of syphilis, but there is little information on such changes.

Skin lesions of secondary syphilis are thought to be a result of immune response accompanied by the presence of APCs and the influx of *T. pallidum*-sensitized T cells produced during the primary stage of infection. Reverse transcription and polymerase chain reaction analyses of syphilitic skin lesions have revealed that these infiltrating T cells produce interferon (IFN)-γ consistent with a Th1 response (10, 11), but the precise localization of IFN-γ$^+$ cells in the skin lesions of secondary syphilis is not known.

In order to determine the roles of CD83$^+$ DCs and IFN-γ$^+$ cells in the formation of skin eruptions in secondary syphilis, we used an immunohistochemical technique to detect CD83$^+$ DCs and IFN-γ$^+$ cells *in situ* at the protein level, and compared the results with those obtained in patients with eczema.

MATERIAL AND METHODS

Specimens and reagents

Five patients with secondary syphilis (all males, mean ± SD age, 49.6 ± 7.1 years) referred to Kyushu University Hospital were investigated. The diagnosis in each case was based on appearance of the skin lesions and the results of non-treponemal and treponemal serologic tests. Moreover, 5 patients with chronic eczema (4 males and 1 female, mean ± SD age, 63.2 ± 11.1 years) were studied. Lesional skin was incisionally biopsied after obtaining informed consent from the patients. Formalin-fixed, paraffin-embedded sections were used for examination in this study.

Mouse monoclonal antibodies against CD83 (clone: HB15A, IgG2b, Immunotech, Marseille, France), CD1a (clone: O10, IgG1, Immunotech), CD68 (clone: KP-1, IgG1, DAKO, Glostrup, Denmark), CD8 (clone: C8/144B, IgG1, Nichirei, Tokyo, Japan), CD4 (clone: 1F6, IgG1, Novocastra Laboratories Ltd., UK) and IFN-γ (clone: 25718.11, IgG2a, Genzyme/ Techne, USA) were used as primary antibodies. Control mouse IgG1, IgG2a and IgG2b were purchased from Pharmingen, San Diego, CA, USA.

Immunhistochemistry

The sections were stained using a Histofine SAB-AP kit (Nichirei, Tokyo, Japan) according to the manufacturer's recommendations. A series of preliminary experiments were done to optimize the antigen-retrieval system. Based on the results, skin sections were treated with 0.1% trypsin for 60 min at 37°C prior to immunostaining for CD83 (12, 13) and IFN-γ (14) as previously reported by us. Heat pretreatment was performed by incubating the sections in 10 mM citrate buffer (pH 6.0) in a pressure cooker at 110°C for 5 min prior to immunostaining for CD68, CD1a, CD4 and CD8. The sections were incubated with primary antibodies overnight at 37°C. We used a new fuchsin for the Histofine SAB-AP kit as a substrate, and the sections were counterstained with hematoxylin. The control isotype-matched mouse IgG1, IgG2a and IgG2b antibodies always showed negative staining. The positive staining cells were enumerated as a percentage of 100 counted dermal infiltrating cells. Five areas were counted and mean ± SD percentage was calculated in each specimen. Data were analyzed using Student's *t*-test; $p < 0.05$ was considered to be statistically significant.

RESULTS

Routine histopathologic examinations in all 5 patients with syphilis showed mixed mononuclear infiltrates, frequently perivascular in distribution, with various numbers of plasma cells, which were consistent with those of secondary syphilis. Samples from patients with syphilis were examined for the presence of immature CD1a⁺ DCs, mostly of the Langerhans' cell type. CD1a⁺ cells (0.65 ± 0.34%) were detected in the upper dermis. To determine the distributions of other APCs, we examined CD68⁺ macrophages. CD68⁺ macrophages (6.33 ± 4.73%) were found in the upper dermis. Mixtures of CD4⁺ cells and CD8⁺ cells were present in the skin lesions. CD8⁺ cells seemed to be dominant. The CD83 antibody used in our study stained some interdigitating cells in an inguinal lymph node. Immunohistochemical staining showed a small but significant subpopulation of CD83⁺ DCs in the upper dermis of secondary syphilis (3.88 ± 3.77%). CD83⁺ DCs were in close contact with lymphocytes (Fig. 1). High-intensity staining of CD83 antigen was detected not only on the surface but also in the cytoplasm of DCs considered phenotypically and functionally activated and mature (Fig. 2). Most CD83⁺ cells were found to be large, irregularly shaped cells with large nuclei and dendrite formation (Figs 1, 2). A small number of mononuclear lymphoid cells (2.60 ± 2.94%) were stained by anti-IFN-γ antibody in the upper dermis (Fig. 3). IFN-γ⁺ cells were relatively uniform in size. The pattern of IFN-γ staining appeared to be intracellular in mononuclear lymphoid cells.

Samples from 5 patients with chronic eczema showed very few CD83⁺ DCs (0.68 ± 0.77 %) and IFN-γ⁺ cells (0.40 ± 0.55%) in the upper dermis of eczema. The difference in the number of CD83⁺ DCs and IFN-γ⁺ cells between syphilis and eczema was not statistically significant. However, the frequency of samples exhibiting more than 1% positive cells was lower in eczema patients than in syphilis patients. In eczema, CD83⁺ DCs (>1%) were found in one of 5 samples, and IFN-γ⁺ cells (>1%) in 2 of 5, $p = 0.048$ and $p = 0.17$, respectively. In syphilis, all samples showed CD83⁺ DCs (>1%) and IFN-γ⁺ cells DCs (>1%). The intensity of the CD83 immunoreactivity in eczema was much weaker than that in secondary syphilis.

Fig. 2. High-intensity staining of CD83 antigen detected not only on the surface but also in the cytoplasm of dendritic cells, which are considered to be phenotypically and functionally activated and mature.
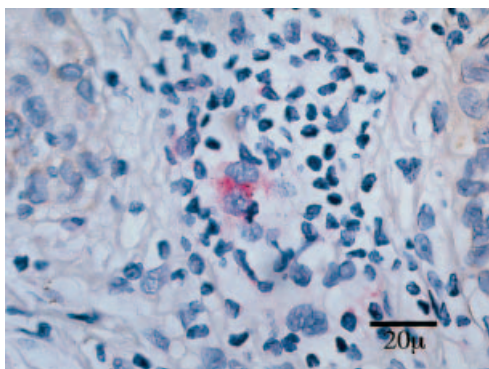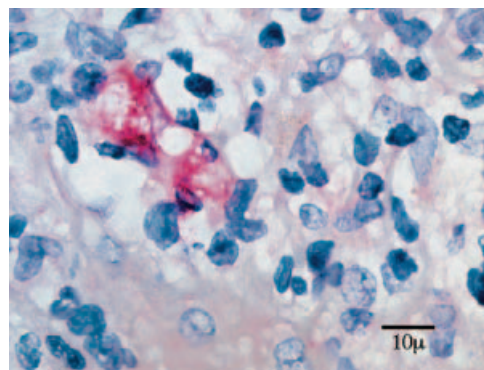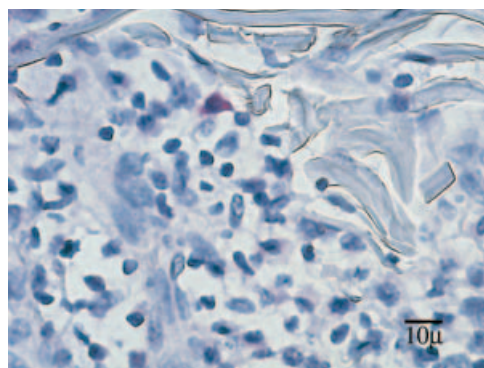

Fig. 1. A small but significant subpopulation of CD83⁺ cells is found in the upper dermis. CD83⁺ cells are in close contact with lymphocytes. CD83⁺ cells are large, irregularly shaped cells with large nuclei and dendrite formation.

Fig. 3. A small number of IFN-γ⁺ cells are observed in the dermis. IFN-γ⁺ cells are relatively uniform in size. The pattern of IFN-γ staining appears to be intracellular in mononuclear lymphoid cells.

DISCUSSION

Recent progress has been made in uncovering the nature and diversity of the interactions between DCs and pathogens and the modulation of the functions of DCs by microbial stimuli (7). In addition to *in vitro* studies on activation of DCs, several groups have reported the ability of microbes and microbe-derived stimuli to influence the immunobiology of DCs *in vivo* (15, 16). Recent studies have characterized the interaction between *T. pallidum* and DCs and provided evidence that treponemal lipoproteins can contribute to the activation of DCs (8, 9). It is important to determine whether this activation is triggered *in vivo* by exposure to *T. pallidum* in patients with syphilis. In this study, we have demonstrated *in vivo* the existence of populations of CD83^{+} DCs in the dermis of patients with secondary syphilis. Furthermore, these CD83^{+} cells were found to be in close contact with lymphocytes, suggesting that communication between these two cell types *in situ* is involved in the immune response at the site. This novel finding indicates that activated and mature CD83^{+} DCs may play a role in immune response in the skin lesions of secondary syphilis.

It is thought that skin lesions of secondary syphilis are caused by immune responses mediated by activated Th1 cells, which are present during the course of the disease within lesional skin (10, 11, 17). Our finding of IFN-γ^{+} cells in patients with secondary syphilis indicates that T-cell response to *T. pallidum* in syphilis infection is biased towards the Th1 phenotype.

It is not clear whether activated and mature CD83^{+} DCs in patients with secondary syphilis are derived from blood DCs, dermal DCs, or epidermal Langerhans' cells. Langerhans' cells are thought to represent a subset of DCs distinguished by their expression of the CD1a antigen, but their exact relationship to CD83^{+} DCs is not fully understood. A considerable number of CD1a^{+} cells were detected in our study, but it seems unlikely that CD83^{+} DCs co-express CD1a. The CD83^{+} DCs may infiltrate the skin lesions of secondary syphilis through the circulation. It is also possible that the activation and maturation of immature DCs occur in skin lesions of secondary syphilis.

In previous studies it has been reported that a small number of CD83^{+} DCs were observed in the skin lesions of sporotrichosis (12) and leprosy (18). In our study, we have demonstrated the existence *in vivo* of a population of CD83^{+} DCs in the skin lesions of secondary syphilis. The CD83^{+} DC is therefore expected to be of importance in the antimicrobial immune response in the skin. CD83^{+} DCs were also present in the epidermis and upper dermis of psoriasis (13).

Immune cell activation by *T. pallidum* is a consequence of its uptake and degradation (19), and such actions would release lipoproteins from subsurface compartments of *T. pallidum*. Lipoproteins are the principal proinflammatory mediators during syphilitic infection, and they efficiently activate various immune effector cells, including DCs (5, 6). A recent study has demonstrated that upon microbial stimulation, Toll-like receptors were recruited to phagosomes of macrophages and were responsible for subsequent activation of the cells (20). It is likely that DCs engulf *T. pallidum* into phagosomes, lipoproteins are liberated, and cell activation occurs via the participation of one or more Toll-like receptors at the level of the phagosome (21).

In conclusion, we have demonstrated the presence of activated and mature CD83^{+} DCs and IFN-γ^{+} cells in skin lesions of patients with secondary syphilis. These CD83^{+} DCs, which are thought to be activated by *T. pallidum* and/or its proinflammatory membrane lipoproteins, may be involved in driving the Th1 response in the skin lesions of secondary syphilis.

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