CD30-Positive Atypical Lymphoid Cells in Common Non-Neoplastic Cutaneous Infiltrates Rich in Neutrophils and Eosinophils

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CD30-positive cells characterize lymphomatoid papulosis and anaplastic large cell lymphoma but can also be found in nonneoplastic skin disorders. Purportedly, CD30 is useful in the differential diagnosis between insect bites and lymphomatoid papulosis. Recently, a subtype of neutrophil-rich CD30-positive anaplastic large cell lymphoma has been described, which may enter the differential diagnosis of cutaneous neutrophil-rich inflammatory infiltrates. We studied atypical CD30-positive lymphoid cells in five eosinophil-rich and 23 neutrophil-rich common nonneoplastic skin infiltrates. The eosinophil-rich cases included five insect bites. The neutrophil-rich cases included nine inflammatory (hidradenitis suppurativa [n = 4], stasis ulcer [n = 2], ruptured cyst, rhynophyma, and Sweet syndrome); 12 infectious (bacterial [n = 8], viral [n = 2] and fungal [n = 2] etiologies); and 2 environmental (spider bites) cases. Atypical CD30-positive cells were found in 4 of 5 eosinophil-rich, 8 of 9 neutrophil-rich inflammatory, 6 of 12 neutrophil-rich infectious, and 2 of 2 neutrophil-rich environmental cases. Polymerase chain reaction analysis of neutrophils, eosinophils, plasma cells, B cells (using CD20), and T cells (using CD3) were performed in the cases that contained atypical CD30-positive lymphoid cells. CD30-positive cells averaged 4.8% of the cells counted in the areas where they were most concentrated. Of the 18 cases that amplified with polymerase chain reaction of T-cell receptor rearrangements; 10 were polyclonal and 8 oligoclonal for B-cell immunoglobulin rearrangements. There was no correlation between B-cell oligoclonality with CD30-positive cell counts, T-cell clonality, or disease category. In conclusion, the presence of CD30-positive atypical lymphoid cells in 71.4% of the common nonneoplastic cases studied, even in the presence of clonal B-cell populations, warrants caution in the interpretation of these cells as malignant, particularly when dealing with the differential diagnosis of lymphomatoid papulosis or neutrophil-rich anaplastic large cell lymphoma.

CD30 is a member of the tumor necrosis factor/nerve growth factor receptor superfamily originally described as a surface molecule recognized by the Ki-1 monoclonal antibody on Hodgkin and Reed-Sternberg cells. Apart from the expression on these cells, the Ki-1 antigen was detected in activated B and T cells but not on resting ones. The Ki-1 antibody did not react in paraffin-embedded tissue, but Ber-H2 was developed to recognize a formalin-resistant epitope within the Ki-1 molecule recognized by the Ki-1 monoclonal antibody on Hodgkin and Reed-Sternberg cells. Physiologically, CD30-positive cells are restricted to a small area in B-cell follicles and do not circulate in the peripheral blood. The role of CD30 in the immune system is not fully understood. CD30 expression on T cells has been proposed as a marker for type 2 helper T cells, as a subset of CD4-positive CD45RO-positive (memory) T cells, and as a nonspecific activation marker for T cells.

Pathologically, the CD30 antigen is not only found on Hodgkin and Reed-Sternberg cells but also on anaplastic large cell lymphoma (ALCL) and lymphomatoid papulosis (LyP). CD30 expression also has been described in cases of mycosis fungoides evolving into large cell lymphoma, and pagetoid reticulosis. Although many papers have been written about CD30-positive lymphomas of the skin, little is known about inflammatory diseases of the skin with expression of CD30.
MATERIALS AND METHODS

We selected 28 cases of common neutrophil-rich and eosinophil-rich nonneoplastic skin infiltrates from the files of the Department of Pathology at the University of South Alabama. The cases were chosen to represent wide disease categories, including inflammatory (n = 9), infectious (n = 12), and environmental (n = 7) conditions. Routinely processed formalin-fixed, paraffin-embedded tissue sections stained with hematoxylin and eosin were examined in all cases. Immunohistochemistry studies for CD3, CD20, and CD30 were done in all cases with monoclonal antibodies from Dako (Carpinteria, CA, USA), using the manufacturer's predilution. Immunohistochemical labeling was performed using standard techniques (labeled streptavidin-biotin and horseradish peroxidase) on 5-μm tissue sections. Antigen retrieval was performed for CD30 using citrate buffer pretreatment with steamer methodology, followed by rinse with Tris-buffered saline (pH 7.6).

Polymerase chain reaction (PCR) studies for B- and T-cell clonality were performed in all the cases that contained CD30-positive cells. DNA was extracted from paraffin-embedded tissue as previously described. B- and T-cell clonality was studied by analyzing IgH and T-cell receptor-β (TCR-β) gene rearrangements, respectively, by PCR using previously published primers. Specifically, the framework 3 VH region and JH consensus sequences were targeted for the IgH gene, and the V1-8, V9, V10, V11, JP, Jp1, Jp2, and J1-2 regions were targeted for the TCR-β gene. All samples were run at least in duplicate using undiluted and diluted template. Polymerase chain reaction results were interpreted as monoclonal when one crisp band was present in neat and diluted samples, polyclonal when a smear was present or when bands were different in neat and diluted samples, and oligoclonal when few distinct matching bands in neat and diluted samples could be visualized after gel electrophoresis. DNA analyses were conducted without knowledge of clinical or pathologic data.

RESULTS

As an initial approach to study this heterogeneous group of 28 common nonneoplastic inflammatory cell infiltrates, the cases were qualified as having a prominence of neutrophils or eosinophils using a 1+ to 3+ semiquantitative scale, in which the group's most prominent infiltrates were qualified as 3+ and the least prominent as 1+. In this fashion, five cases were classified as eosinophil rich and all corresponded to insect bites (Fig. 1A). The rest of the cases were classified as neutrophil rich and included diseases from the inflammatory, infectious, and environmental categories (Fig. 2A). In this neutrophil-rich group we included a case of genital herpes and one of hidradenitis suppurativa (case nos. 12 and 20), which in addition to the neutrophilic infiltrates contained prominent eosinophils. This may be related to the fact that these two cases occurred in HIV-positive patients. In this manner, the neutrophil-rich category included nine inflammatory conditions, including hidradenitis suppurativa (n = 4), stasis ulcer (n = 2), ruptured cyst, rhinophyma, and Sweet syndrome; 12 infectious conditions, including bacterial (n = 8), viral (n = 2), and fungal (n = 2) etiologies; and 2 environmental conditions represented by spider bite cases (Table 1).

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<th>Table 1</th>
<th>Clinical information, semiquantitative assessment of inflammatory cells, and PCR studies for T- and B-cell clonality in cases that contained CD30+ cells</th>
<th>ER, eosinophil-rich; NR, neutrophil-rich; -E, environmental; -I, infectious; -R, inflammatory; Neu, neutrophils; Eos, eosinophils; CD3, T-cells; CD20, B-cells; CD30, atypical CD30+ cells; BC, B-cell clonality; TC, T-cell clonality; M, monoclonal; P, polyclonal; O, oligoclonal; NA, no amplification; ND, not done.</th>
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<td>FIG. 1</td>
<td>Eosinophil-rich case corresponding to an insect bite (case no. 4). (A) Numerous eosinophils are noted in this low power examination (original magnification ×40). (B) In the same field numerous CD30-positive atypical cells are noted. (C) On high power examination the atypical cells demonstrate large nuclei and prominent nucleoli (arrows). Several eosinophils, lymphocytes, and plasma cells are also noted in this field (original magnification ×160). (D) The large atypical lymphoid cells are positive for CD30 with a membrane/Golgi pattern.</td>
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<td>FIG. 2</td>
<td>Neutrophil-rich case corresponding to a perirectal abscess (case no. 8). (A) Numerous neutrophils are noted in this low power examination (original magnification ×40). (B) In the same field numerous CD30-positive atypical cells are noted. (C) On high power examination, the atypical cells demonstrate large nuclei and prominent nucleoli (arrows). Several neutrophils are also noted in this field (original magnification ×160). (D) The large atypical lymphoid cells are positive for CD30 with a membrane/Golgi pattern.</td>
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We then assessed the presence of atypical CD30-positive cells, T and B lymphocytes (using CD3 and CD20), in the eosinophil-rich and neutrophil-rich groups, using the semiquantitative approach already described (Figs. 1B and 2B). The CD30-positive atypical cells in both the eosinophil-rich and neutrophil-rich groups were medium to large and had abundant amphophilic cytoplasm and large nucleus with prominent nucleolus (Figs. 1C and 2C). The pattern of CD30 immunoreactivity in the atypical cells was membranous and Golgi (Figs. 1D and 2D). Plasma cells had diffuse CD30 cytoplasmic immunoreactivity different from the atypical lymphoid cells and were not considered in the counts (Fig. 3). Atypical CD30-positive cells were found in 8 of 9 neutrophil-rich inflammatory cases, 6 of 12 neutrophil-rich infectious cases, 2 of 2 neutrophil-rich environmental cases, and 4 of 5 eosinophil-rich cases (Table 1). The CD30-positive cells were more prevalent in the environmental and inflammatory categories and less prevalent in the infectious category. This may be explained by the more acute nature of the inflammatory cell infiltrate present in the infectious cases examined, which generally contained fewer B and T cells (Table 1).

Cell counts of neutrophils, eosinophils, plasma cells, B cells (using CD20), and T cells (using CD3) were performed in the 20 cases that contained CD30-positive cells. We counted these cells in a single ×40 high power field (area 0.22 mm²) that had the highest density of CD30-positive cells. We counted an average of 806 cells (range 162-2224) per high power field. Then we established the relative percentage of each cell population (Table 2). The average percentage of CD30-positive cells was 4.8 (range 0.3-25.6%) of the total cells counted in the particular high power field where they were the most concentrated. The CD30-positive cells were mostly associated with T cells (51.0%) and B cells (22.6%). Neutrophils and eosinophils, although prominent in most of the lesions, were not particularly abundant in the fields where the CD30-positive cells were most concentrated (Table 2).

Of the 20 cases containing CD30-positive atypical lymphoid cells, 18 cases amplified successfully with PCR for T- and B-cell clonality (Figs. 4 and 5). All cases were polyclonal for TCR rearrangements. Regarding B-cell immunoglobulin rearrangements, 10 cases were polyclonal and eight cases were oligoclonal (Table 1). Polyclonal and oligoclonal cases were not correlated to CD30-positive cell counts, a particular disease, or a disease category.

**FIG. 3.** CD30 immunoreactivity of atypical lymphoid cells and plasma cells (case no. 21). CD30-positive atypical lymphoid cell immunoreactivity with a membrane/Golgi pattern (vertical arrow) can be differentiated with the diffuse cytoplasmic CD30 immunoreactivity in plasma cells (horizontal arrow) (original magnification ×160).

**TABLE 2.** Quantitative assessment of inflammatory cells in the 40× high-power field (area: 0.22 mm²) richest in CD30+ cells **The results are expressed as the average percentage of cells in each disease category.** ER, eosinophil-rich; NR, neutrophil rich (-E, environmental; -I, infectious; -R, inflammatory); Neu, neutrophils; Eos, eosinophils; PC, plasma cells; CD3, T-cells; CD20, B-cells; CD30, atypical CD30+ cells.

**FIG. 4.** T-cell clonality PCR studies (representative cases). Samples were tested for TCR-Î³ gene rearrangements and loaded on a 4% agarose gel as follows: lanes 1 and 2, Jurkatt cell line (monoclonal control); lanes 3 and 4, case no. 20, neat and diluted (polyclonal); lanes 5 and 6, case no. 1 (polyclonal); lanes 7 and 8, case no. 4 (polyclonal); lane 9, negative control; lane 10, molecular weight markers.

**FIG. 5.** B-cell clonality studies (PCR, representative cases). Samples were tested for IgH gene rearrangements and loaded on a 4% agarose gel as follows: lane 1, molecular weight markers; lane 2, Raji cell line (monoclonal control); lanes 3 and 4, case no. 23, neat and diluted (polyclonal); lanes 5 and 6, case no. 20 (oligoclonal); lanes 7 and 8, case no. 24 (polyclonal); lanes 9 and 10, case no. 10 (oligoclonal); lanes 11 and 12, case no. 27 (oligoclonal); lanes 13 and 14, case no. 21 (oligoclonal).

**DISCUSSION**

Here we demonstrate the presence of atypical CD30-positive lymphoid cells in a group of common nonneoplastic cutaneous infiltrates rich in neutrophils and eosinophils, chosen to represent a wide variety of reactive inflammatory, infectious, and environmental conditions. The CD30-positive atypical lymphoid cells were found in the setting of a mixed inflammatory reaction containing both T and B lymphocytes. The CD30-positive cells were more prevalent in the environmental and inflammatory categories and less prevalent in the infectious category.
All cases studied were polyclonal for TCR rearrangements. The majority of cases were also polyclonal for B-cell immunoglobulin rearrangements. Interestingly, a proportion of cases also had oligoclonal B-cell populations. There was no significant correlation of B-cell clonality with the numbers of CD30-positive cells, B cells, with a particular disease, or disease category. Sparse reactive B cells in extranodal tissues such as the skin can result in clonally restricted band patterns evidenced as oligoclonality. Even in the presence of monoclonality, these false-positive bands observed in the setting of sparse B-cell populations should not be used as evidence for B-cell neoplasia. 13

Altogether the immunophenotypic and genotypic results of our study indicate that the CD30-positive cells are a component of a reactive rather than a neoplastic process. In a good proportion of inflammatory settings, we were able to demonstrate high concentrations of CD30-positive cells with a characteristic immunoactivity pattern. In our illustrations, one can observe a typical Golgi and membrane labeling that must not be interpreted as evidence of lymphoproliferative disease. 19 In addition, we found concentrations of CD30-positive atypical cells of up to 25.6% of all the cells counted in a given high power field in a spider bite biopsy (case no. 6). Even faced with this high density of CD30-positive atypical cells, one must be careful before interpreting this sole finding as a lymphoproliferative disorder. 11

The presence of CD30-positive atypical lymphoid cells in nonneoplastic settings is particularly relevant in the differential diagnosis of LyP and the neutrophil-rich variant of ALCL. In 1992, Smoller et al. studied CD30 expression in LyP and insect bite reactions. 21 These authors found that all of the LyP cases had scattered CD30-positive atypical lymphoid cells in the dermis and virtually no labeling with CD30 in the insect bite reactions, despite the presence of atypical large lymphocytes. 20 Our finding of atypical CD30-positive cells in the majority of insect bites invalidates the assumption that CD30-positive cells are useful in excluding LyP in this setting. The lack of immunoreactivity for CD30 in the Smoller et al. study 21 may be the result of different sensitivity of the monoclonal antibody or, most likely, the lack of use of antigen retrieval methodology in their study. 20

In 1995, Mann et al. described a neutrophil-rich variant of CD30-positive ALCL. 10 They described six cases of ALCL that had neutrophils in proportions varying from 5% to >50% of the total cells. Of the six patients, three had skin lesions. 10 More recently, Jhala et al. reported two cases of neutrophil-rich ALCL that presented as scalp masses in HIV-positive men and pointed out that this rare morphologic variant of ALCL should be considered in the histologic evaluation of neutrophil-rich biopsy specimens. 7 Our finding of atypical CD30-positive lymphoid cells in a large proportion of nonneoplastic neutrophil-rich infiltrates warns against interpreting this sole finding as lymphoma. Of note, two of the patients (case nos. 12 and 20) in our study were HIV positive. These patients had a particularly prominent increase in CD30-positive cells. This may be related to the known upregulation of CD30, a marker of type 2 helper T-cell cytokine environments, in the setting of HIV infection. 9

In summary, atypical CD30-positive lymphoid cells are frequently found in common cutaneous nonneoplastic inflammatory cell infiltrates rich in neutrophils and eosinophils. This can potentially cause differential diagnostic problems with CD30-positive lymphoproliferative disorders. On the basis of these findings, it is obvious that the presence of CD30-positive atypical cells or of IgH gene rearrangements in a lymphomatoid infiltrate of the skin does not qualify it for being considered malignant. 11 The differentiation between benign and malignant cutaneous lymphoid infiltrates is only possible through the combined use of multiple clinical, histologic, immunophenotypic, and molecular genetic analyses, as there is no single reliable criterion that would allow this distinction. 3 Large atypical CD30-positive lymphoid cells are a part of common reactive infiltrates and do not qualify alone for the diagnosis of malignancy.

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Keywords:
CD30; Lymphoma; Inflammation; PCR; HIV

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Inflammatory Dermatopathology: The Diagnosis of Cutaneous Inflammatory Infiltrates With Atypical CD30-Positive Cells.
Kahn, Andrea G. MD; Horenstein, Marcelo G. MD
[Abstract] [Fulltext] [PDF (1.23 M)]