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CD30 expression on CD1a+ and CD8+ cells in atopic dermatitis and correlation with disease severity

Atopic dermatitis (AD) is a chronic inflammatory skin disease associated with cutaneous hyperreactivity to environmental stimuli, resulting in increased infiltration of inflammatory cells, IgE production and enhanced expression of costimulatory molecules, cytokines and chemokines. CD30, a TNF receptor superfamily member, is a costimulatory molecule expressed on activated T and B cells. A positive correlation between soluble CD30 (sCD30) levels in patient serum and AD disease severity has been described previously. However, the relative frequencies and identities of cells expressing CD30 in AD patients and the relationship between the frequency of CD30 positive cells and serum sCD30 levels with disease severity remained unknown. To address these questions, immunofluorescence analysis of AD skin lesions representing different disease stages, was conducted. In addition to the CD4+ T cells, CD1a+ Langerhans cells and CD8+ T cells were found to express CD30 in AD lesions and the cell numbers correlated with disease severity. FACS analysis of AD patient blood samples revealed expression of CD30 on memory T-cells and a correlation with disease severity was identified. Finally, serum analysis of soluble mediators revealed positive correlations between sCD30, IgE, MDC, TARC and PARC levels with disease severity. Combined, our data provide correlative evidence that CD30+ cells, including Langerhans cells and CD8+ T-cells, may contribute to AD disease severity and that therapeutic strategies targeting CD30+ cells may provide benefit to AD patients.

Key words: CD30, atopic dermatitis, Langerhans cells, CD8 T cells, chemokine

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Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease that often precedes respiratory allergy. It is a worldwide health problem, affecting 5-20% of children [1]. Many factors have been implicated to contribute to the development of inflammation and severity in AD, including genetic, environmental, dermatologic, pharmacologic, psychological and immunologic factors [2]. The diagnosis of AD is based on the presence of associated clinical features, including severe pruritus, a chronically relapsing course, typical morphology and distribution of the skin lesions, as well as a personal or family history of atopic disease [3, 4]. Most patients with AD display elevated levels of serum IgE and increased numbers of circulating eosinophils, reflecting an increased expression of Th2 cytokines and chemokines [2]. Various forms of therapy, including corticosteroids, UV therapy and immunosuppressants, are currently being used in the management of this complex disease [2, 5]. Despite progress in the treatment options, moderate to severe AD remains an unmet medical need and new approaches are greatly needed.

The leukocyte activation marker CD30 is a member of the TNF-R3 superfamily and was originally identified based on its strong expression on Reed-Sternberg cells in Hodgkin's

disease [6]. Increased CD30 expression was subsequently found to be associated with different types of malignancies and clonal inflammatory disorders such as lymphomatoid papulosis [7]. The expression of CD30 in normal cells is restricted to activated T- and B-cells but was found absent on resting lymphocytes or monocytes. CD30 is expressed at high levels on activated lymphocytes in various forms of autoimmune diseases and is detectable in the circulation of patients with rheumatoid arthritis [8], multiple sclerosis, and systemic sclerosis [9, 10].

Several groups investigating the role of CD30 in AD have reported increased levels of sCD30 in the serum of AD patients relative to non-atopic controls [11-15]. In contrast, no increase in sCD30 levels were found in patients with allergic contact dermatitis (ACD) or psoriasis [11, 12, 16], a finding that may reflect differences in the etiology between AD and ACD. Similar to the differences in serum levels, high proportions of CD30+ and CD4+ skin-infiltrating cells were reported in AD skin lesions [13, 15], whereas virtually no CD30+ cells were found in patients with ACD [13, 17]. In the blood of AD patients, a subpopulation of peripheral CD30+ cells, defined as CD30+ CD4+ CD45RO+ cells, were found to be present at significantly

increased numbers, which was not found in non-atopic controls [18]. Combined, these findings suggest that the increase in serum sCD30 levels in AD patients may reflect its release by infiltrating CD30+ cells or by circulating CD30+ T cells. However, the relationship between the numbers of CD30+ cells and disease severity as well as the identity of cells expressing CD30 in AD skin lesions remain unknown.

In this report we establish correlations between disease severity and both soluble and cell-bound CD30. In addition, we identified CD1a+ Langerhans cells and CD8+ T cells to express CD30 in atopic dermatitis lesions and explored the relationship between serum sCD30 and chemokine levels. Among the latter, monocyte derived chemokine (MDC), thymus activated and regulated chemokine (TARC) and pulmonary activated and regulated chemokine (PARC) [19] were previously shown to correlate with disease activity [20-26]. Our studies identified correlations between serum levels of these chemokines and sCD30 levels in AD patients and further validate sCD30 as a potential diagnostic tool in AD indications.

Methods

Patients

The criteria of Rajka-Langeland [3, 4] were used for the diagnosis of AD. Thirty patients with AD and 10 healthy controls were enrolled in the study after providing informed consent. The investigational protocol was approved by the Ethics Committee of the Oregon Health Sciences University. AD patients ceased all atopic dermatitis-specific therapy, such as topical steroids and topical calcineurin inhibitors as well as antihistamines, at least one week before enrollment. Blood samples and skin biopsies were obtained from all subjects, including 10 healthy, 10 mild, 10 moderate and 10 severe AD based on Rajka-Langeland baseline assessment [3]. For immunohistological analysis, 5-mm punch biopsies were generally obtained from most active eczematous skin lesion. The biopsies were embedded in OCT Tissue-Tek (Sakura Finetek USA Torrance, CA), and stored at - 80 °C until further processing.

Immunohistochemical staining

Frozen tissue sections were prepared and stored as described above for the immunohistochemical staining procedure. To determine the distribution and the number of CD4+, CD8+, CD30+ and CD40+ cells in lesional skin sections, single immunostainings were performed at Phenopath Laboratories. Briefly, to prevent nonspecific protein binding, antibodies were mixed with 1% BSA in PBS (Invitrogen, Carlsbad, CA) and applied overnight to frozen sections in a humid chamber at 4 °C. Primary antibodies including, CD4, clone RPA-T4, (BD Pharmingen, San Jose, CA), diluted 1:50, CD8, clone C8/144B, (DakoCytomation, Carpinteria, CA), diluted 1:100, CD30, clone BerH2, (DakoCytomation, Carpinteria, CA), diluted 1:40, CD40, clone 5C3, (BD Pharmingen, San Jose, CA), diluted 1:500 was used. Envision+ Mouse HRP Polymer Detection System was used (DakoCytomation, Carpinteria, CA). After washing the slides with PBS with 0.05% Tween, DAB+ Substrate (DakoCytomation, Carpinteria, CA) one drop chromogen per each 1.0 ml Substrate Buffer was used. The

procedure was followed by Mayer's hematoxylin for counterstain and 0.5% ammonia water for blue staining of nuclei. After two steps incubation with ethanol 95% and 100% and xylene, the slides were mounted under coverslips and were read in a blinded fashion at 100 × to 400 × magnification, using a light microscopy (Carl Zeiss MicroImaging, Inc., Thornwood, NY). Three to five pictures were taken from individual lesions and the CD8, CD40 and CD30+ cells were counted per field. The average numbers of cells were determined by dividing the total cell count with the numbers of the fields analyzed.

Flow cytometry

Peripheral blood samples were processed within 24 hours following collection. Wright-stained smears were prepared; complete blood cell counts were performed on a Beckman-Coulter ActT Diff2 cell counter. 250,000 to 500,000 leukocytes were aliquoted into a round-bottom polystyrene tube in a volume of 50 µL, and incubated with an optimized cocktail of fluorescently-conjugated monoclonal antibodies for 15 minutes at room temperature in dark conditions. The antibodies used included: CD4-PE-CY7 (Beckman-Coulter, Hialeah, FL clone SFC112T4D11), CD45RO-FITC, (Beckman-Coulter, Hialeah, FL, clone UCHL1), CD30-APC (Caltag/Invitrogen, Burlingame, CA, clone HRS-4). Following antibody incubation, 1.5 mL of lyse/fix reagent (ammonium chloride/ultrapure formaldehyde) was added directly to the tube and incubated for an additional 15 minutes at room temperature in the dark to lyse the red blood cells. Following centrifugation, the cells were washed once in 3 mL of solution of phosphate buffered saline (PBS)/1% bovine serum albumin (BSA)/0.01% sodium azide. Following a second centrifugation, the cells were resuspended in approximately 250 µL of PBS/BSA/azide. Cells were analyzed on the same day they were processed, using a BD LSRII flow cytometer. Typically, 100,000 to 150,000 viable leukocytes were acquired per specimen, and the flow cytometry data were analyzed using BD's FACS Diva software, version 4.1.2.

Immunofluorescence staining

Frozen tissues were cut into 5-µm-thick sections and mounted on capillary gap microscope slides. The cryostat sections were air-dried for 20 min, fixed in ice-cold acetone for 10 min and stained immediately. To determine the phenotype, distribution and the numbers of antigen presenting cells (APCs) in lesional and healthy skin, single or multi-color immunostaining was performed. The monoclonal antibodies used in the study were CD4-Alexa Fluor 488, CD8-Alexa Fluor 647, CD1a-Alexa Fluor 647, CD11b-Alexa Fluor488 (Biolegend, San Diego, CA), goat-anti-human-IgG- Alexa Fluor 568, (Molecular Probes, Eugene, OR). Anti human CD30 antibody (cAC10) (Seattle Genetics Inc., Bothell, WA) and mouse anti human CD3 (Biolegend, San Diego, CA) was conjugated with Alexa Fluor 568, (Molecular Probes, Eugene, OR) at Seattle Genetics by the antibody drug conjugate group. Triple immunofluorescence staining was performed as follows: antibodies (5 µg/mL) were mixed with 1% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO) in PBST (Invitrogen, Carlsbad CA) and applied to frozen sections in a humidified chamber at room temperature for 1 hr. After washing with PBS, slides were incubated for 5 minutes with DAPI (0.5 µg/mL, Molecular Probes, Eugene, OR) to

visualize the nucleus. After this step, slides were washed and mounted in Vectashield Mounting Medium (Vector Laboratories, Burlingame, Calif., USA). Pictures were taken with a fluorescence microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY) equipped with a filter for double or triple staining, at 100× to 400× magnification.

Serum analysis

Serum sample analysis of sCD30, IgE, MDC, TARC and PARC was conducted by Pierce's SearchLight Multiplex Sample Analysis service, utilizing the SearchLight Multiplex Array System (Pierce Biotechnology, Cambridge, MA). This system is a multiplex sandwich ELISA (enzyme-linked immunosorbent assay) in a planar, plate-based array format, for the quantitative measurement of secreted proteins in serum. Briefly, each well of the microplate is pre-spotted with analyte-specific antibodies. These antibodies captured specific proteins in the standards and samples added to the plate. After unbound proteins are washed away, the biotinylated detecting antibodies were added and bind to a second site on the target proteins. After washing away excess detecting antibody, streptavidin-horseradish peroxidase (SA-HRP) was added. The HRP enzyme was subsequently allowed to react with the substrate, SuperSignal ELISA Femto Chemiluminescent Substrate (as described in Patent 6,432,662) to produce a luminescent signal that was detected using the SearchLight CCD Imaging and Analysis system. The amount of signal produced was proportional to the amount of each protein in the original standard or sample. Customized Array Vision software utilizes a weighted four parameter curve fit to back-calculated unknowns. The results were then sent back to Seattle Genetics. Graphing the results and statistical analysis was conducted using the linear regression analysis provided in the Graphpad Prism Software Package version 4.01 (Graphpad, San Diego, CA).

Results

CD30 expression levels correlate with AD disease severity

Previous studies have described a positive correlation between increased sCD30 serum levels and the severity of AD (mild, moderate and severe). To verify and extend on these findings, we included patients ranging from AD severity index 3 to 9 using the Rajka-Langeland criteria [3, 4] in our analysis. Determination of serum sCD30 levels revealed a moderate but statistically significant correlation ($r^2 = 0.3310$, $p = 0.0009$) with the disease severity index, confirming previous reports (figure 1A). We further explored the relationship between *in situ* CD30 expression and disease severity. Immunohistochemical (IHC) analyses of skin lesions from patients revealed almost complete absence of CD30 expression in healthy skin sections, and a gradual increase in CD30+ cells in AD skin lesions, which correlated with disease stages (figure 1B $p = 0.0001$) and severity index (figure 1C; $r^2 = 0.3908$, $p = 0.0002$). In addition, there was a moderate but significant correlation between the mean numbers of CD30+ cells in AD skin lesions and serum sCD30 levels ($r^2 = 0.2622$, $p = 0.0038$) (figure 1D). Circulating CD4+ memory T cells were previously described to express CD30 in AD patients [18]. However,

there is no information available regarding the expression of CD30 on CD8+ memory T cells. In this report, we analyzed the percentage of memory T cell populations, shown as CD4+CD45RO+CD30+ or CD8+CD45RO+CD30+ cells. We found a significant increase in both types of CD30+ memory T cell populations in the blood of severe AD patients relative to healthy controls and positive correlations with disease progression were established (figures 1E and F).

Serum sCD30 levels correlate with AD disease severity

Serum levels of MDC, TARC and PARC were previously shown to be increased in AD and to correlate with disease severity [20-26]. To extend on these observations, we investigated the relationship between these chemokine levels and serum sCD30. When analyzing patient serum samples, we found that MDC, TARC and PARC levels correlated significantly with serum sCD30 levels ($r^2 = 0.3310$, $p = 0.0009$), ($r^2 = 0.4314$, $p < 0.0001$), ($r^2 = 0.2831$, $p = 0.0025$), respectively (figures 2A, B and C). Serum IgE levels were reported to be elevated in AD patients [1]. When examining the serum IgE levels, we found a less pronounced but statistically significant correlation between serum sCD30 and serum IgE levels ($r^2 = 0.1660$, $p = 0.0254$) (figure 2D).

CD30 is expressed on CD8+ T cells

CD4 positive T cells are known to play important roles in AD pathogenesis [13, 15]. In our study we identified an increase in CD4+ T cell infiltration that correlated with disease severity (figure 3A). However, the relative number of CD4 T-cell infiltrates exceeded the numbers of CD30 positive cells in the same lesions (figures 1C and 3B). Furthermore, when conducting double staining experiments for CD4 and CD30, we found that CD30 expression was only detectable on a small proportion of lesional CD4+ cells (figures 4C and D). These findings suggested that the marked increase in CD30 positive cells in AD lesions may be associated with infiltration of different inflammatory cell types. In order to identify such additional cell types expressing CD30, we conducted immunofluorescence staining of skin sections using antibodies detecting CD8 and CD30. We found most CD8+ T cells co-stained for CD30 (figures 4E and F). Furthermore, we performed IHC studies and quantified CD8 positive cells in skin sections from healthy controls and AD patients. Our studies revealed an almost complete absence of CD8+ cells in healthy skin sections (figure 5B). In sections from AD patients, CD8+ T cells were mostly located in the dermis region and were present at lower levels relative to CD4+ T cells (figure 5A). We found a gradual increase in the numbers of CD8+ cells in the skin of mild, moderate and severe atopic dermatitis patients (figure 5B) and a weak but statistically significant correlation between the numbers of CD8+ cells and the disease severity index ($r^2 = 0.1732$, $p = 0.0221$) (figure 5C). In support of expression of CD30 on CD8+ cells, we observed a significant correlation between the cell numbers expressing these markers ($r^2 = 0.3167$, $p = 0.0012$) (figure 5D).

CD30 is expressed on CD1a+ cells within AD skin lesions

We noticed that some of the CD30+ cells located in the epidermis of AD skin lesions displayed a typical Langer-

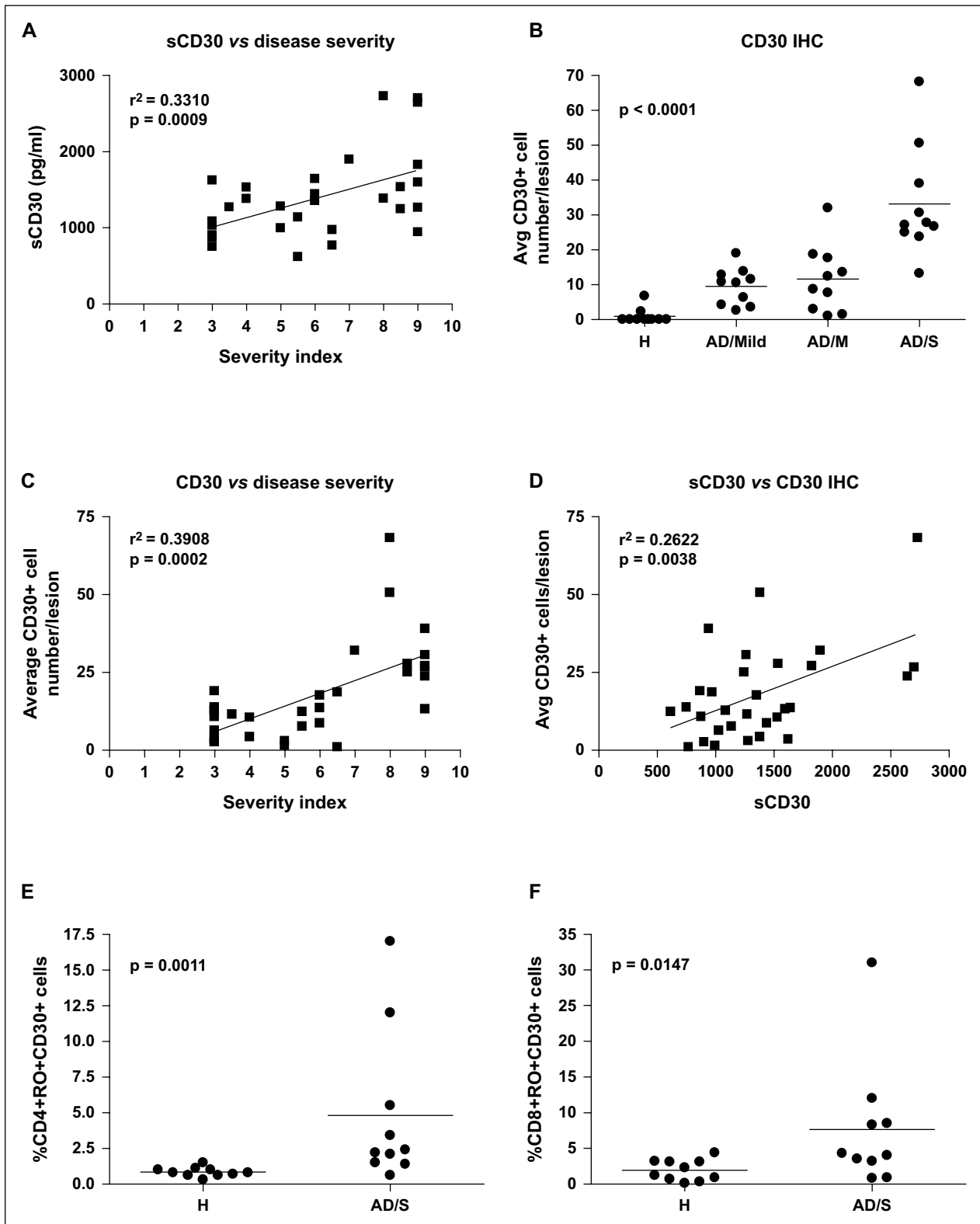


Figure 1. CD30 expression correlates with disease severity in AD. **A)** Correlation between sCD30 levels and AD disease severity index. Levels of sCD30 were determined using a sandwich ELISA SearchLight Multiplex Array System. **B)** Average CD30+ cell number for healthy controls (H, $n = 10$), for patients with mild disease activity (AD/Mild, $n = 10$), moderate disease activity (AD/M, $n = 10$), severe disease activity (AD/S, $n = 10$) as assessed by IHC analysis of CD30 in lesional biopsies **C)** Correlation between average CD30+ cell numbers per lesion and disease severity index ($n = 30$) **D)** Correlation between average CD30+ cell numbers per lesion and serum sCD30 levels ($n = 30$). **E)** FACS analysis of CD4+CD45RO+CD30+ cells in peripheral blood and **F)** CD8+CD45RO+CD30+ cells in blood of healthy (H, $n = 10$) and patients with severe disease activity (AD/S, $n = 10$).

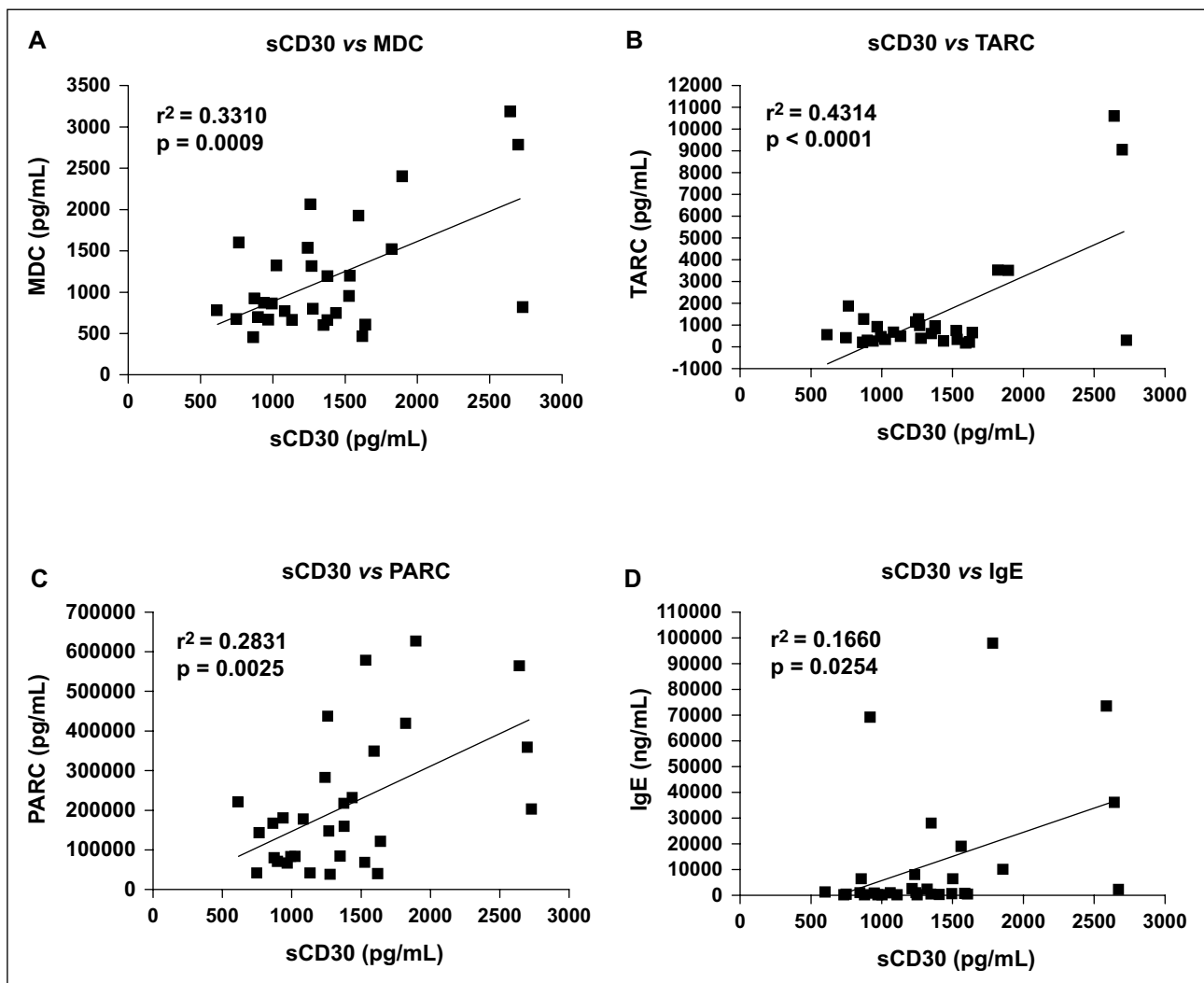


Figure 2. Serum sCD30 levels correlate with cytokine and serum IgE levels in AD samples. Serum levels were determined using a sandwich ELISA SearchLight Multiplex Array System ($n = 30$). Linear regression analysis was performed to determine the correlation between serum levels of sCD30 and **A.** MDC, **B.** TARC, **C.** PARC and **D.** IgE.

hans cell morphology (*figure 6A*). In order to better determine the identity of these cells, we co-stained the sections with antibodies recognizing CD1a (*figure 6B*) and CD11b, representing markers for Langerhans and myeloid cells, respectively. While CD11b+ cells were mostly negative for CD30 expression (data not shown), almost all of the CD1a+ cells were positive for CD30 (*figure 6C*). In addition, we immuno-histochemically stained AD skin lesions for CD40, a surface antigen expressed on activated dendritic cells. When comparing the numbers of CD30+ with the numbers of CD40+ cells, we identified a significant correlation ($r^2 = 0.5273$ $p < 0.0001$) between both markers in skin lesions of AD patients (*figure 6D*).

Discussion

In this report, we determined the levels of selected serum chemokines and sCD30 levels in patients with atopic dermatitis and found correlations with AD disease severity. We also found correlations between sCD30 and MDC, TARC,

PARC, IgE levels in the serum of AD patients. Finally, we demonstrated that CD30 is expressed on CD8+ T cells as well as CD1a+ Langerhans cells. Similar increases in infiltrating CD30+ inflammatory cells within affected tissues were found to be associated with several immunological disorders, including systemic lupus erythematosus, scleroderma, measles virus, HIV infection, allergic rhinitis, asthma and AD [8-18]. Among the various cell types displaying increased CD30 expression in AD patients, several were proposed to play important roles in regulating inflammation in AD, in particular CD4+T cells [11-18]. Here we report expression of CD30 on two additional, less well characterized types of inflammatory cells in AD patients: CD8+ T-cells and CD1a+ antigen presenting Langerhans cells. It is worth noting that despite the correlative evidence provided in this report, the role of CD30 positive cells in the initiation and progression of AD disease remains unclear.

CD30, a TNF receptor family member, is expressed as a transmembrane glycoprotein and its extracellular domain can be cleaved proteolytically to produce the soluble form (sCD30), which was found to be released from cells propa-

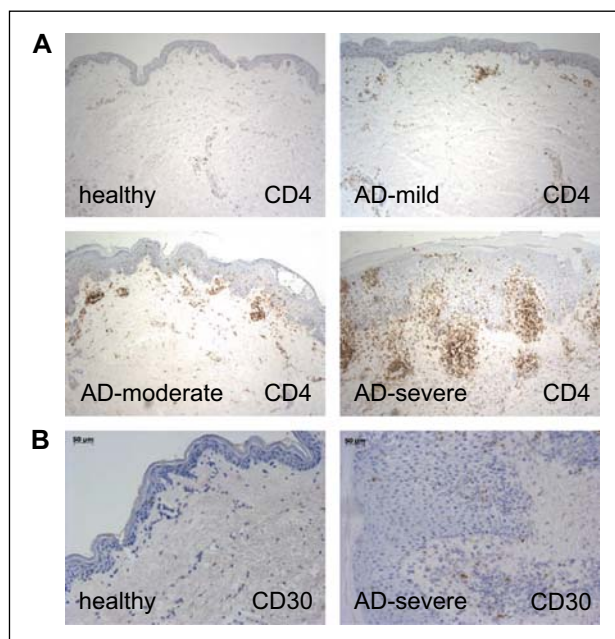


Figure 3. Skin infiltrating CD4+ T cells increase with AD disease severity. Immunohistochemical staining for **A.** CD4 positive cells in skin from healthy, mild, moderate and severe AD patients (100× magnification) **B.** CD30 positive cells in skin from healthy and severe AD patients (200× magnification).

gated *in vitro* or *in vivo*. The utility of CD30+ cells present in tissue sections or sCD30 levels in the serum of AD patients as potential biomarkers in AD indications has been studied and discussed previously [12-15, 18, 27, 28]. In this report, we describe a previously unknown correlation between serum sCD30 levels and the numbers of CD30+ cell in skin lesions as well as a correlation between CD30+ cells in lesional skin and disease severity. For diagnostic purposes, the quantification of CD30 tissue levels by IHC may not be ideal because of the invasive nature of skin biopsies. In contrast, peripheral blood specimens are preferred for diagnostic purposes because of their minimally invasive nature. These findings further support the use of sCD30 not only as a prognostic marker for AD, but eventually as a predictive marker for response to treatment or to monitor the effects of therapeutic compounds, pending the validation of a causative role of CD30+ cells in AD disease initiation and progression. Our findings thus support the use of blood samples for correlative studies in AD.

Our study conducted with 30 AD patients helped to better characterize the phenotype of CD30+ cells found within the skin lesions of AD patients. Previously, CD30 expression was reported on CD4+ T cells in skin sections and on CD4+ memory T cells in PBMCs [13, 17, 18]. Correlative clinical studies investigating a potential correlation between disease severity and the presence of CD30CD8 double positive cells have not been reported. In this study, we observed markedly lower numbers of CD30+ (*figure 3B*) when compared with CD4 expressing cells (*figure 3A*), which were more comparable to the numbers of CD8+ cells (*figure 5A*). These findings prompted us to examine which subset of T-cells co-express CD30. Double staining of AD skin section revealed that CD30 was predominantly expressed on

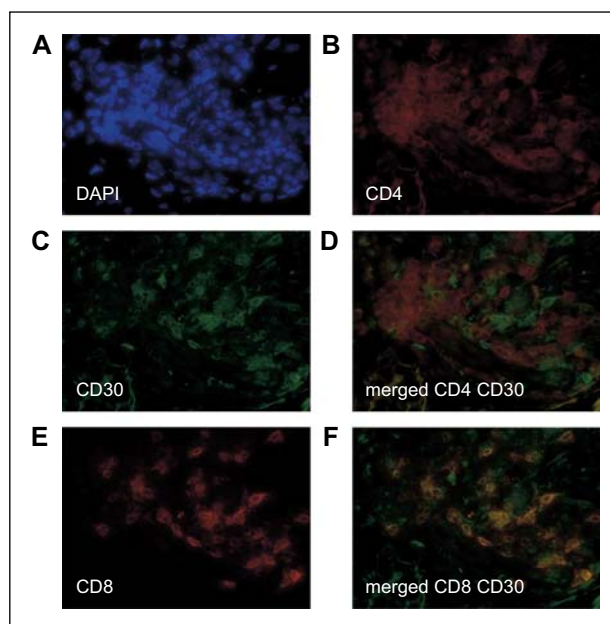


Figure 4. CD30 is expressed on CD8+ cells. Immunofluorescence staining of severe AD skin lesions with **A.** DAPI staining (blue) for visualizing nuclei **B.** mouse-anti human CD4 (red) **C.** Anti human CD30 (green) **D.** the merged images, mouse-anti human-CD4 (red), mouse anti-CD30 antibody (green), CD4CD30 double positive (yellow), **E.** mouse-anti human CD8 (red), **F.** the merged images, mouse-anti human-CD8 (red), mouse anti-CD30 antibody (green), CD8CD30 double positive (yellow). All pictures were taken from the dermis section of the skin with 400× magnification.

CD8+ T cells located in the dermis region. Finally, we found that CD30 is expressed on CD8+ memory T cells in the blood of AD patients (*figure 1F*).

The contribution of the different cell types expressing CD30 to the initiation and progression of AD remains to be determined. Preclinical studies in models of autoimmune disease suggested key regulatory roles for either cell type during inflammation. CD8+ T-cells were shown to be present in atopic dermatitis [29] and to contribute to progression of other atopic diseases, including asthma [30, 31]. In the epidermal region of AD lesions, we detected CD30 expression on CD1a+ Langerhans cells. The role of these cells in AD disease progression remains unknown, however, CD1a+ Langerhans cells were shown to exert important functions in several preclinical models of inflammatory diseases [32, 33], including psoriasis [34, 35]. In AD patient lesions, CD1a+ cells have been studied extensively and the increased presence of intraepidermal Langerhans cells as well as inflammatory dendritic epidermal cells (IDEC) have been reported [32, 36].

A recent study by Fischer *et al.* revealed that the CD30-CD30L interaction also plays a critical role in stimulating mast cells in the absence of antigen, and mast cell activation has been suggested to occur during AD development. These findings further support the importance of the CD30/CD30L receptor and ligand interaction in atopic disease [37]. In conclusion, interference with CD30/CD30L interactions may be a promising strategy for anti-inflammatory therapy in AD as it may affect several key cell types involved

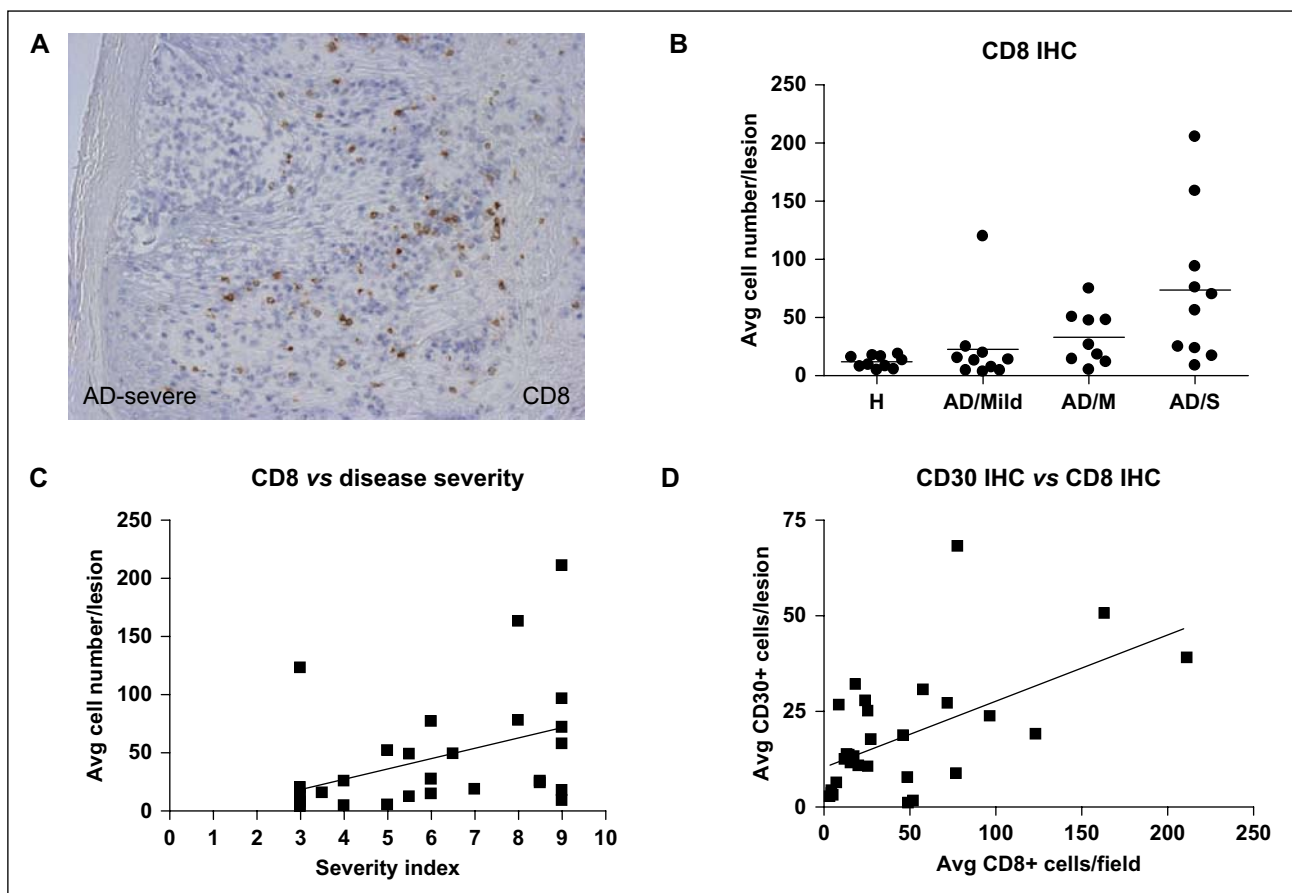


Figure 5. Increased numbers of infiltrating CD8+ T in severe AD patients. Immunohistochemical staining of **A**. CD8+ cells in skin from severe AD patients (200X) **B**. Average CD8+ cell number in skin sections of healthy controls (H, n = 10), and patients with mild (AD/Mild, n = 10), moderate (AD/M, n = 10) and severe disease activity (AD/S, n = 10). **C**. Linear regression analysis to determine the correlation of average CD8 + cell numbers and disease severity index (n = 30). **D**. Linear regression analysis to determine the correlation between CD8 + cell numbers and CD30+ cell number in AD skin lesions (n = 30).

in inflammatory responses, including, CD4+ T-cell, CD8+ T-cell, CD1a+ cells, memory T-cells and mast cells.

CD30 is a co-stimulatory molecule that interacts with CD30L, which is expressed predominantly on T cells and mast cells. This interaction has been shown to be important for activation of both cell types. Antigens such as dust mite and bacterial products (*i.e.* LPS) become engulfed and presented by Langerhans cells located within the epidermis of the skin, ultimately triggering T-cell responses [2]. Subsequent to antigen stimulation of T cells, CD1a+ cells become activated and trigger an inflammatory response by producing chemokines including MDC, PARC, TARC and pro-inflammatory cytokines (*i.e.* TNF- α). These soluble mediators stimulate effector T-cells to produce IL-4, which is a required costimulatory signal along with CD40/CD40L to activate B-cells to produce IgE [20, 24, 38]. Finally, IgE binding to Fc ϵ Re expressed on mast cells triggers histamine production and the release of inflammatory molecules contributing to the inflammatory cascades. The model of the inflammatory cascade described above may help to explain the correlation between CD30 levels in the skin lesions of AD patients and the serum chemokine and IgE levels identified.

Improved therapies inducing minimal toxicity for the treatment of moderate to severe AD are greatly needed. A CD30 receptor blockade or the elimination of CD30+ cells represent potential therapeutic strategies as they may interrupt several pathogenic processes operative in AD inflammation, including activation and antigen presentation by dendritic cells, chemokine secretion, T cell and mast cell activation. In experimental clinical studies conducted in various autoimmune indications, co-stimulatory proteins have been targeted individually by strategies including targeting TNF-alpha family members, resulting in promising initial responses [39]. The expression of CD30 on cells critically involved during inflammatory processes suggests that targeting CD30 may result in therapeutic benefit in AD patients. In conclusion, our data provide additional evidence for the potential utility of sCD30 as a serologic marker to determine disease severity and provide further evidence that CD30 represents a promising therapeutic target in AD. ■

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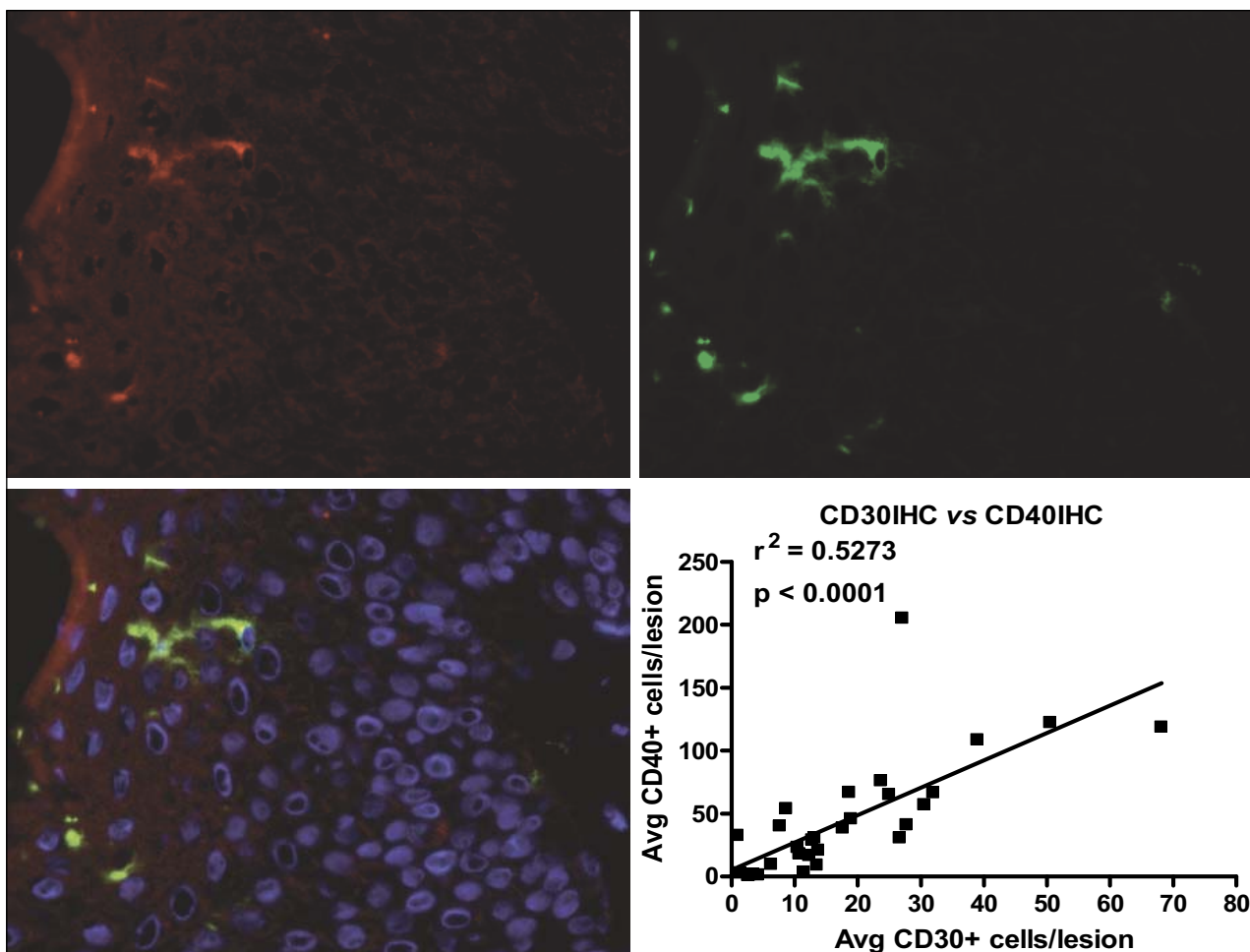


Figure 6. CD1a+ cells express CD30 in AD. Immunofluorescence staining of severe AD skin lesions with **A.** Anti human-CD30 antibody (red) **B.** Anti human-CD1a antibody (green) **C.** the merged images, human-CD1a (green), human CD30 antibody (red), CD1a CD30 double positive (yellow) and DAPI staining of nuclei (blue). All pictures were taken from epidermis section of skin with 400× magnification. **D.** Linear regression analysis was performed to determine the correlation between CD30+ cells and CD40+ cells in AD skin lesions (n = 30).

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