Comparison of CD30, HLA-DR and CD23 expression in bronchial biopsies obtained from patients with atopic asthma and sarcoidosis

BARBARA ROGALA', ANDRZEJ GABRIEL', JERZY KOZIELSKI2, SŁAWOMIR ŻEGLEŃ3

1Chair and Clinical Department of Internal Diseases, Allergology and Clinical Immunology, Medical University of Silesia; 2Department of Pneumonology, Medical University of Silesia; 3Department of Pathomorphology, Medical University of Silesia

Abstract

Th2-type cells play a crucial role in the regulation of inflammatory process in atopic disorders. In contrast, the importance of Th1-type response during granulomatous inflammation is postulated. There is some evidence that CD30 cell surface molecule is associated with the differentiation/activation pathway of human Th2-type cells.

The aim of this study was to evaluate the spontaneous expression of CD30 in comparison with the other cell activation markers - HLA-DR, CD23 - in bronchial biopsy specimens recovered from patients suffering from atopic bronchial asthma or sarcoidosis.

For this purpose 13 patients with episodic atopic bronchial asthma during asymptomatic period of the disease (FEV1 >80% predicted) diagnosed according to ATS criteria were included into the trial. The bronchial mucosa slides obtained from 5 patients with sarcoidosis were studied.

A few biopsy specimens of bronchial mucosa and submucosa were taken from a peripheral bronchus of the inferior lobe during the fiberoptic bronchoscopy. The deparaffinized sections were processed in three stages method (ABC) using mouse monoclonal antibody labelled by isotype specific biotinylated rabbit anti-mouse immunoglobulins and subsequently by streptavidin-peroxydase complex amplified. Studies were evaluated semiquantitatively.

In patients with asthma the CD 30+ mononuclear cells in the epithelium (slight and moderate positive reaction) in 38% of patients were observed. The expression of CD23 on mononuclear cells in the epithelium (slight positive reaction) in only three subjects was noticed. In contrast, strong and moderate HLA-DR expression in all specimens studied both in the epithelium and in submucosa was observed. In patients with Sarcoidosis there was moderate positive reaction with CD30 in three patients. Like in asthma strong positive reaction of HLA-DR antigen was observed in all subjects. CD23 was slightly and moderately positive in three patients.

We conclude that:

1. CD30+ cells cannot be considered as an useful differentiative marker between bronchial asthma and sarcoidosis.
2. It cannot be excluded that some stages of inflammation during sarcoidosis are Th-2-dependent.
3. The spontaneous increased expression of HLA-DR molecule in bronchial biopsy specimens from asymptomatic asthmatics and sarcoid patients during remission support the notion that mucosal inflammation persists in the airways irrespectively to the stadium of the diseases

Key words: asthma, sarcoidosis, Th-2, Th-1, CD30, HLA-DR, CD23

**Introduction**

Th2-type cells are commonly known as playing a crucial role in the pathophysiology of atopic asthma. Th-2 type cytokines IL-4 and IL-5 play a central role in the inflammatory response that characterizes asthma in both the atopic and nonatopic forms of the disease [1, 2].

It is commonly ascertained that sarcoidosis is caused by exposure to an environmental, possibly infectious, agent and that there may be genetic susceptibility to the disease. Despite the tools of modern medicine the cause of sarcoidosis remains unknown. Granulomatous inflammation is regulated by a complex interplay of T cells, mononuclear phagocytes, fibroblasts, B cells, dendritic cells [3, 4].

Many studies support the notion and clearly demonstrate an imbalance between Th-1 and Th-2-like activity in sarcoidosis with suppression of Th-2 and increase in Th-1 [5]. The administration of corticosteroids to patients with sarcoidosis restores the balance between locally produced Th-1 and Th-2 cytokines and immunoglobulin isotypes to normal.

It was clearly demonstrated that Th-1/Th-2 lymphocytes subpopulations balance is based on secreted cytokines profile. Notwithstanding there is some evidence that CD30 cell surface molecule, a member of TNF (tumor necrosis factor)/NGF (nerve growth factor) receptor superfamily is associated with the differentiation/activation pathway of human T cells also of these producing Th2-type cytokines [6-11].

The expression of CD30 in bronchoalveolar lavage in interstitial lung diseases was comparable with the control group, additionally no significant correlation with Th-2 characteristic cytokine – IL-5 was observed [12].

HLA-DR antigens belonging to Class II Human Leukocyte Antigens is a product of MHC (Major Histocompatibility Complex) genes, HLA-DR expression enter into the composition of typical immunohistological dendritic cells, macrophages and B cell features. The increased HLA-DR expression observed during allergic inflammation may be associated with the presentation of antigens to T cells and/or with activation state of the cell [13-15].

Process of cell activation/presentation is performed with participation and/or transmitted among the other cytokines by interleukine 4 (IL-4) of a great importance which increases the expression not only of class II MHC molecule but also of the low affinity IgE-Fc receptor – FcεRII identified with CD23. CD23 is known to be located on peripheral activated lymphocytes, eosinophils and platelets. Macrophages demonstrate intensified expression of Fc(RII) receptor in atopic individuals increasing cells ability to antigen’s presentation [16].

The aim of the presented work was to evaluate the spontaneous expression of CD30 as well as HLA-DR, CD23 in bronchial biopsy specimens recovered from patients suffering from atopic bronchial asthma in comparison to sarcoid subjects.

**Material**

1. 13 patients (7 men, 6 women ranging in age from 20 to 41 years, mean age 27.4) with episodic atopic bronchial asthma during asymptomatic period of the disease (FEV1 >80% predicted) diagnosed according to ATS criteria were included into the trial. All subjects were allergic as judged by positive skin response to common inhalant allergens.

   Inclusion criteria were as follows: mild asthma with FEV1 greater than 70% of predicted value, degree of bronchi reversibility more than 12%, total serum IgE levels more than 100 kU/l. They all had positive skin prick test responses, defined as a wheal 3 mm greater than that caused by physiologic saline control to one or more inhaled allergens. (diagnostic set – Allergopharma).

   Exclusion criteria: severe asthma with FEV1 lower than 70% of predicted value, degree of bronchi reversibility less than 12%, lack of atopy markers – total serum IgE levels less than 100 kU/l, negative skin prick test responses, steroidotherapy and/or immunotherapy in preceding period (3 months steroidotherapy, 3 years immunotherapy), current or previous smoker, respiratory tract infection during the month preceding the test.

2. The slides of bronchial mucosa obtained from 5 patients with sarcoidosis (3 men, 2 women ranging in age from 19 to 41 years, mean age 29.5) were studied. The diagnosis of pulmonary sarcoidosis was based on histological examination of biopsy samples of the lung. Clinical and radiological evaluation were performed to assess the activity of the disease process.

   Excluding criteria: 3 months steroidotherapy in preceding period, current or previous smoker, respiratory tract infection during the month preceding the test.

**Methods**

Premarkedication with Atropine (0.5 mg s.c.), Codeine (0.02 g p.o.) was administered. During the procedure oxygen supplementation was applied. The larynx and upper airways were anesthetized with Xylocaine 2 percent spray. The oxygen saturation was monitored with a digital oxymeter. A few biopsy specimens of bronchial mucosa and submucosa were taken from a peripheral bronchus of the inferior lobe during the fiberoptic bronchoscopy (Bronchoscope Olympus, Tokyo).

The biopsies were fixed in buffered 5% paraformaldehyde for 24-48 hrs in 20°C then embedded in paraffin and cut into 5 µ slides. The preparations had been counterstained with hematoxylin prior to the execution of immunohistochemical reaction.

Immunohistochemical analysis was made by ABC method (Avidin – Biotin – Complex Kit Duet Dako, Copenhagen) using monoclonal antibodies for following antigens:
1. CD30 (N1558 DAKO, Denmark and LSAB 2 kit DAKO KO 677),
2. CD23 (NCL-1B12 dilution 1:200, Novocastra UK and Strept AB complex/HRPDuet, Mouse/Rabbit, DAKO),
3. HLA-DR (NCL-LN3, dilution 1:200, Novocastra, UK and Strept AB complex/HRPDuet, Mouse/Rabbit, DAKO).

Reactions were visualized by means chromogen DAB (diaminobensidine). Before immunostaining for detection CD 23 antigen slices were boiled (90°C under high pressure during 5 minutes). Normal tonsil tissue served as the immunohistochemical staining control for all monoclonal sera.

Histopathological preparations were assessed under a microscope (BX-40, Olympus, Japan) with magnification ranging from x 40 to x 400.

The intensity of each reaction was assessed under x 400 magnification semiquantitatively using a grading scale defined as:

- **+** - less than 5 positive cells in the field of vision - slight positive reaction,
- **++** - less than 10 and more than 5 positive cells in the field of vision - moderate positive reaction,
- **+++** - more than 10 positive cells in the field of vision - strong positive reaction.

Cells whose cytoplasm was intensively stained with a dark brown color were assessed as causing a positive reaction. Independent teams each consisting of two diagnosticians performed immunohistochemical markings Simmons.

Morphometric analysis for cells expressing HLA-DR was not performed as the assessment of positive reaction in this method is subject to high percentage of error. The analysis of the immunohistochemical reaction allows percentage assessment of positive reaction calculated in a high field vision, magnification x 400 (analysis between 5 and 10 fields) or number of positive reactions per 1000 cells representing a homogenous population. In this study biopsies included different lymphocyte sub-populations. Statistical analysis might not applied, because in this study only a small number of bronchial mucosa biopsies was included.

The study was performed after informed consent was given by patients and after Local Ethical Committee approval.

**Results**

**Asthma**

CD30+ mononuclear cells in the epithelium (slight and moderate positive reaction) in five out of thirteen patients were observed. In one case disseminated infiltrations CD30+ mononuclear cells under the epithelium were observed. A second instance showed single positive stained cells and focally diffused infiltrations. The remaining subjects presented single positive lymphocytes under the epithelium in the basal layer.

The expression of CD23 on mononuclear cells in the epithelium (slight positive reaction) was noticed only in three subjects. Single infiltrations were observed within the epithelium borders.

In contrast, HLA-DR expression in all specimens studied both in the epithelium and in submucosa was observed. The expression of HLA-DR molecule was strongly positive not only on mononuclear cells but also on dendritic cells. HLA-DR molecules were identified both on cellular surface and within the cells.

The presented slides show strong positive reaction with HLA-DR antigen (photo 2) and slight positive reaction with CD30 (photo 1).

The level of studied antigens expression is presented on table 1.

**Table 1.** The expression of membrane antigens in subjects with asthma

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>CD30</th>
<th>CD23</th>
<th>HLA-DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>negative</td>
<td>negative</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>++</td>
<td>negative</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>negative</td>
<td>negative</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>++</td>
<td>negative</td>
<td>+++</td>
</tr>
<tr>
<td>5</td>
<td>negative</td>
<td>negative</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>negative</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>++ single infiltrations of lymphocytes under the epithelium</td>
<td>negative</td>
<td>++</td>
</tr>
<tr>
<td>8</td>
<td>+ single in epithelium</td>
<td>+ in epithelium</td>
<td>+++</td>
</tr>
<tr>
<td>9</td>
<td>negative</td>
<td>negative</td>
<td>+++</td>
</tr>
<tr>
<td>10</td>
<td>negative</td>
<td>negative</td>
<td>++</td>
</tr>
<tr>
<td>11</td>
<td>negative</td>
<td>+ on MNC, in the epithelium</td>
<td>+++</td>
</tr>
<tr>
<td>12</td>
<td>negative</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>13</td>
<td>negative</td>
<td>negative</td>
<td>+++</td>
</tr>
</tbody>
</table>

Barbara Rogala et al.
Comparison of CD30, HLA-DR and CD23 expression in bronchial biopsies obtained from patients with atopic asthma and sarcoidosis

Sarcoidosis

There was moderate positive reaction with CD30 molecule in samples from three subjects with single positive lymphocyte clearly seen in submucosa.

Here also as in the case of asthma, HLA-DR expression was strong in all specimens studied. Reaction was visualized both in the epithelium and in submucosa.

Slight and moderate positive reaction with CD23 in three patients was observed. The reaction intensification was comparable with CD30 expression in slides obtained from patients with asthma. Single positive stained cells and focally diffused infiltrations were seen.

The level of studied antigens expression is presented on table 2.

Discussion

CD30 molecule expression is observed as well in asthmatic bronchial mucosa as in sarcoid tissue

Should CD30 membrane molecule be considered as the marker of Th-2 type lymphocytes or as a functionally important membrane molecule with defined function in the cell activation and differentiation?

Asthma

CD30 as the marker of Th-2 lymphocytes. Data related to CD30 molecule and Th-2-type subpopulation cells are confusing. There is some evidence that CD30 cell surface molecule is associated with the differentiation/activation pathway of human T cells producing Th2-type cytokines [17]. However there are data suggesting that induction of CD30 on activated T cells is not related to differentiation into T cells subpopulations and does not discriminate between human Th1 and Th2 type T cells [18, 19].

According to Romagnani [11] CD30 should be considered as the marker of Th-2 type lymphocytes. The high level of CD30 expression in atopic asthmatics is thought to be proof of the important role of Th-2 type cells in the pathophysiology of atopy. CD30 ligand-CD30 interaction has been shown to positively influence development of the Th-2 phenotype [20].

Due to our results we could not exclude CD30 as a functionally important molecule in pathophysiology of atopy but for sure it should not be considered as the marker of atopic constitution.

A soluble form of CD30 (sCD30) is released by CD30+ cells in vivo [21]. High levels of HLA-DR expression on

Table 2. The expression of membrane antigens in subjects with sarcoidosis

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>CD30</th>
<th>CD23</th>
<th>HLA-DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>negative</td>
<td>negative</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>++</td>
<td>negative</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>negative</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>5</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Photo 1. Moderate positive reaction with CD30 (5-10 positive cells in the field of vision), immunohistochemical staining, bronchial asthma, magn. 400x

Photo 2. Strong (diffused) positive reaction with HLA-DR (more than 10 positive cells in the field of vision), immunohistochemical staining, bronchial asthma, magn. 200x
Sarcoidosis

peripheral CD30+ cells and by the presence of high levels of serum soluble interleukin-2 receptor and sCD30 was associated with Fas gene alleles and lack of Fas-induced apoptosis was observed in patients with autoimmune lymphoproliferative syndrome (ALPS) [22]. Some authors indicated that a high level of sCD30 is associated with lower detectable levels of CD30L on their PBLs in patients with CD30 tumors. Next, CD30L takes part in mediation of apoptosis. Blocking membrane-bound CD30L mediated apoptosis by sCD30 in the CD30+ cell line (lymphocytes) may lead to disturbances in immunological surveillance [23] – “escape” of lymphocytes from apoptosis. Many factors could influence the maintenance of the described parameter.

We should expect a probable direct relationship between the degree of positive reaction with CD30 as marker of Th-2 type cells and specific and non-specific IgE production stimulated by IL-4 and IL-5 secreted from Th-2 cells. Increased exposure to house dust mite allergen, Der. p. was suggested that development of lymphocytes toward Th-2 type cells is related to different presentation of the same antigen by different APC underlying their fundamental regulatory role. Through stimulation of Th-2 or Th-1 related cytokines secretion, APC are able to indirectly influence inflammatory response in “shock organ” such as the bronchial tree. Atopic asthma considered as Th-2 type related disease differs from sarcoidosis associated rather as Th-1 predominance disorder [5].

The spontaneous increased expression of HLA-DR molecule in bronchial biopsy specimens from asymptomatic asthmatics and sarcoid subjects observed in our study support the notion that mucosal inflammation persists in the airways irrespectively of the asthma symptoms and confirm the continuous status of lymphocytes T activation also observed by other authors both in the bronchial mucosa and bronchoalveolar lavage (BAL) [30, 31].

CD23 molecule in studied biopsies

The role or significance of CD23 in pathogenesis of both atopic asthma and sarcoidosis is still unclear.

Membrane CD23 differentiation antigen identical to low affinity receptor for IgE cells takes the principal role as an adhesion molecule in the process of antigen presentation by lymphocytes. Stimulation of FcεRII expression on monocytes was associated with the induction of IL-4, granulocyte-macrophage-CSF, IFNγ, INFα and macrophage-CSF but not of IL-2, IL-6, or TNFα [32]. There were two forms of IgE receptors: FcεRIIa located on B lymphocytes and EBV infected B cells and FcεRIIb observed on IL-4 – treated B cells and monocytes and B cells obtained from atopic subjects [16]. We could suppose that this antigen should be associated with IgE-dependent mechanism of allergy. It was hypothesized that high levels of IgE to specific and nonspecific allergens often found in asthmatic patients would be associated with expression of low affinity receptors for IgE FcεRII/CD23 in bronchial mucosa.

We performed our study with asymptomatic individuals. There exists the possibility that only during exacerbation...
of the disease this molecule could give more evident positive reaction.

However, some authors, using BAL, observed localized CD23 induction in the asthmatic lung (much greater expression of CD23 on alveolar macrophages than peripheral blood monocytes) irrespective of the disease stage [32]. To answer this incompatibility we should compare expression of CD23 in bronchial mucosa and bronchoalveolar lavage since some differences could exist.

Maybe poor expression of this antigen provides future encouragement for authors who believe in the great importance of T cells rather than in IgE sensitization during atopic inflammation.

Here also as in the case of CD30 antigen we did not notice any correlation between presence of Fc(RII/CD23 antigen in the bronchial tree where the inflammatory process occurred and the concentration of IgE in plasma (data not presented).

Conclusions

1. CD30+ cells cannot be considered as a useful differentiative marker between bronchial asthma and sarcoidosis.
2. It cannot be excluded that some stages of inflammation during sarcoidosis are Th-2-dependent.
3. The spontaneous increased expression of HLA-DR molecule in bronchial biopsy specimens from asymptomatic asthmatics and sarcoid patients during remission support the notion that mucosal inflammation persists in the airways irrespectively to the stadium of the diseases.

References

Partial characterization of soluble Ki-1 antigen and detection of the antigen in cell culture supernatants and in serum by an enzyme-linked immunosorbent assay. Eur J Immunol 19: 157-162.


