Modulation of Accessory Molecules on Lung T Cells*

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Stimulation of T cells via the CD3/TCR complex is associated with the reduced expression of accessory molecules, a phenomenon called modulation. To investigate whether the modulation is implicated in the pathogenic mechanism of pulmonary disease, we determined the MFI of CD3, CD4 and CD8 on lung and blood lymphocytes in ten normal subjects and 54 patients with various pulmonary and extrapulmonary diseases. Although there were no significant differences in MFI of these accessory molecules on lung lymphocytes among the groups, MFI of CD3 on lung lymphocytes was significantly decreased compared with that on blood lymphocytes in all groups, demonstrating the modulation of CD3 on lung lymphocytes. Since it has been shown that T cells whose CD3/TCR is modulated are hyporesponsive to various mitogenic signals, our observation seems to represent another aspect of the mechanism whereby the local pulmonary milieu maintains the autoregulation of immune response. (Chest 1990; 97:1393-1400)

TCR = T cell antigen receptor; CD = cluster of differentiation of (number) molecule; MHC = major histocompatibility complex; AM = alveolar macrophage; IPF = idiopathic pulmonary fibrosis; CVD = collagen vascular disease; TNF = tumor necrosis factor; BALF = bronchoalveolar lavage fluid; BAC = bronchoalveolar cells; FITC = fluorescein isothiocyanate; PE = phycoerythrin; PBS = phosphate-buffered saline; FCS = fetal calf serum; MFI = mean fluorescence intensity; PBS/azide = PBS containing 0.1 percent sodium azide

The important role played by T cells in the local milieu of the lung is well established. There recently have been tremendous advances in our understanding of the nature of the T cell surface molecules required for antigen recognition and subsequent activation and proliferation of T cells. The best studied of these is the CD3/TCR complex. Under physiologic conditions, antigen is presented in the context of self-MHC by antigen presenting cells. A ternary complex involving CD3/TCR, class I or 2 MHC molecules, and nominal antigen is the fundamental structure of antigen recognition by T cells. In addition, it has been proposed that CD4 and CD8 molecules stabilize this structure and account for the restriction of CD4+ helper/inducer T cells with class 2 MHC, and CD8+ suppressor/cytotoxic T cells with class 1 MHC.

After ligand binding to CD3/TCR on T cells, a decrease in the expression of CD3 from the cell surface via endocytosis ensues, a phenomenon called modulation. It has been demonstrated that this down-regulation of CD3 is associated with a concomitant decrease of membrane CD4 and CD8. The modulation of these "accessory molecules" induced by treatment with monoclonal antibodies, in turn, renders T cells unresponsive to antigen or mitogen for a certain period of time. Thus, the extent of the modulation of surface molecules on T cells could reflect the strength of previous antigenic stimulation and the capacity to respond to additional stimulation. Although it generally has been held that the lung is persistently exposed to airborne antigens, and that AM have the ability to present antigens to lung T cells, as yet little is known about the modulation of accessory molecules on lung T cells recovered by BAL.

In this investigation, we addressed this issue by undertaking studies in which we determined the surface densities of CD3, CD4 and CD8 on blood and lung lymphocytes using monoclonal antibodies and flow cytometers. These studies clearly show that CD3 molecules on lung T cells are strongly modulated compared with those on blood T cells. This phenomenon is accompanied by comodulation of CD4 and is commonly seen in both healthy subjects and patients with disease.

METHODS

Study Population

Control subjects and patients with the following five disease categories were evaluated. Detailed demographic data and the results of pulmonary function tests are presented in Table 1, and results of BAL in Table 2.

Normal Subjects

This study population consisted of ten normal individuals. None had a history of lung disease or evidence of lung disease by physical examination, chest roentgenography and pulmonary function tests. All had visibly normal airways within reach of the fiberoptic bronchoscope.

Pulmonary Sarcoidosis

The diagnosis of pulmonary sarcoidosis was established in 26 untreated patients. Each had a compatible clinical picture without evidence of mycobacterial, fungal or parasitic infection, and compatible chest radiographic findings including bilateral hilar and/or
Table 1—Clinical and Physiologic Characteristics of the Study Populations

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Age (yr)</th>
<th>Male/Female</th>
<th>Smokers/Nonsmokers</th>
<th>VC↑</th>
<th>FEV₁%</th>
<th>Dco↑</th>
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<tbody>
<tr>
<td>Normal subjects</td>
<td>10</td>
<td>39 ± 11</td>
<td>5/5</td>
<td>5/5</td>
<td>109±19</td>
<td>84±4</td>
<td>107±7</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>26</td>
<td>41 ± 16</td>
<td>12/14</td>
<td>5/21</td>
<td>109±11</td>
<td>80±10</td>
<td>96±20</td>
</tr>
<tr>
<td>IPF</td>
<td>6</td>
<td>60 ± 5</td>
<td>5/1</td>
<td>5/1</td>
<td>107±17</td>
<td>79±4</td>
<td>82±17</td>
</tr>
<tr>
<td>Interstitial pneumonia with CVD</td>
<td>7</td>
<td>55 ± 11</td>
<td>4/3</td>
<td>5/2</td>
<td>100±14</td>
<td>82±5</td>
<td>61±23</td>
</tr>
<tr>
<td>Other pulmonary</td>
<td>6</td>
<td>51 ± 21</td>
<td>4/2</td>
<td>4/2</td>
<td>92±16</td>
<td>72±9</td>
<td>79±24</td>
</tr>
<tr>
<td>Extrapulmonary</td>
<td>9</td>
<td>39 ± 19</td>
<td>5/4</td>
<td>3/6</td>
<td>101±18</td>
<td>87±13</td>
<td>95±19</td>
</tr>
</tbody>
</table>

*Values are mean ± SD.
†Expressed as percent predicted.
§p<0.01 compared with normal subjects.
$\text{p}<0.05$ compared with normal subjects.

paratracheal lymph node enlargement with or without parenchymal infiltrates. Seventeen patients had biopsy evidence of noncaseating epithelioid cell granulomas. By chest roentgenographic staging, five were in stage 0, 12 in stage 1, eight in stage 2 and one in stage 3.

**Idiopathic Pulmonary Fibrosis**

Six patients fulfilled clinical and radiographic criteria for IPF without evidence of other interstitial lung diseases that potentially cause pulmonary fibrosis. All had transbronchial lung biopsies showing varying degrees of interstitial fibrosis.

**Interstitial Pneumonia Associated with CVD**

A diagnosis of interstitial pneumonia caused by CVD was made in seven patients with diffuse interstitial patterns on chest roentgenograms, serologic evidence of CVD and compatible clinical manifestations. Two had systemic lupus erythematosus, three had rheumatoid arthritis, one had mixed connective tissue disease and one had Sjögren's syndrome. Each fulfilled the established criteria of CVD.*1"17

**Other Pulmonary Disease**

This disease category consisted of six patients with various pulmonary diseases. One had pulmonary lymphangiomatosis associated with tuberous sclerosis, one had interstitial pneumonia associated with bullous pemphigoid, one had interstitial pneumonia and permeability edema due to the administration of TNF for the treatment of renal cell carcinoma, one had eosinophilic pneumonia, one had subpleural curvilinear shadows of unknown etiology and one had pneumoconiosis. Corticosteroids had been administered in patients with bullous pemphigoid, TNF-induced pneumonia and pneumoconiosis.

Miscellaneous Extrapulmonary Disease

This study population consisted of nine patients with various extrapulmonary diseases. Four had Crohn's disease, one had myelofibrosis, three had uveitis and one had cutaneous T cell lymphoma. Corticosteroids had been administered in patients with myelofibrosis and in two patients with Crohn's disease.

**Preparation of BAL Cells and Blood Lymphocytes**

BAL was performed as previously described.18 The recovered BAC were washed three times with Hanks' balanced salt solution (Gibco, Grand Island, NY), and resuspended in autologous serum at a concentration of 5 × 10⁶ cells/ml to establish a condition similar to that of blood lymphocytes. Blood was collected in a tube containing heparin from each subject before BAL.

In some experiments, lung and blood T cells were isolated by rosetting BAC or blood mononuclear cells with neuraminidase-treated sheep red blood cells*9 and suspended in autologous serum at the same cell concentration as that of BAC.

The differential count of cells in BALF was obtained from cytocentrifuged preparations stained with May-Grünwald-Giemsa.

**Immunofluorescence**

Fluorescein FITC-conjugated or PE-conjugated monoclonal antibodies, T₃ (anti-CD3), T₄ (anti-CD4) and T₈ (anti-CD8) were purchased from Coulter Immunology (Hialeah, FL).

In single-color flow cytometric analysis, 100 μL of heparinized blood or BAC suspension placed in 16 × 10 mm plastic tubes (Spitze S, Erma, Tokyo) was incubated with an appropriate antibody for 30 min at 4°C in the dark. Two milliliters of 1 percent lysing

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Table 2—BAL Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cells (×10⁶/ml BALF)</th>
<th>AM (%)</th>
<th>Lymphocytes (%)</th>
<th>Neutrophils (%)</th>
<th>Eosinophils (%)</th>
<th>CD3↑ (%)</th>
<th>CD4↑ (%)</th>
<th>CD8↑ (%)</th>
<th>CD4/8 (%)</th>
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</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>26±34</td>
<td>89±11</td>
<td>10±11</td>
<td>0±0</td>
<td>0±0</td>
<td>82±9</td>
<td>54±14</td>
<td>37±16</td>
<td>2.1±1.5</td>
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<tr>
<td>Sarcoidosis</td>
<td>17±10</td>
<td>65±20†</td>
<td>33±19$</td>
<td>1±1</td>
<td>0±0</td>
<td>88±6</td>
<td>74±11$</td>
<td>16±9$</td>
<td>6.4±4.9$</td>
</tr>
<tr>
<td>IPF</td>
<td>27±21</td>
<td>88±10</td>
<td>7±7</td>
<td>2±3</td>
<td>2±4</td>
<td>86±11</td>
<td>52±24</td>
<td>37±22</td>
<td>2.2±1.9</td>
</tr>
<tr>
<td>Interstitial pneumonia with CVD</td>
<td>29±30</td>
<td>60±37</td>
<td>39±38$</td>
<td>1±1</td>
<td>1±2</td>
<td>87±11</td>
<td>60±24</td>
<td>36±19</td>
<td>2.6±2.5</td>
</tr>
<tr>
<td>Other pulmonary</td>
<td>24±27</td>
<td>67±21</td>
<td>24±15</td>
<td>6±12$</td>
<td>2±3</td>
<td>86±12</td>
<td>45±14</td>
<td>43±14</td>
<td>1.2±0.8</td>
</tr>
<tr>
<td>Extrapulmonary</td>
<td>15±10</td>
<td>68±23</td>
<td>31±23</td>
<td>1±1</td>
<td>0±0</td>
<td>82±13</td>
<td>56±18</td>
<td>34±21</td>
<td>2.8±2.7</td>
</tr>
</tbody>
</table>

*Values are mean ± SD.
†Percent of BALF lymphocytes.
$\text{p}<0.05$ compared with normal subjects.
$\text{p}<0.01$ compared with normal subjects.
solution (Immuno-Lyse, Coulter Immunology, Hialeah, FL) was then added to each tube. The samples were incubated for 5 min and washed twice with PBS/azide. After removal of the supernatant, the cells were resuspended in 400 μL of PBS/azide and immediately analyzed on an Epics C flow cytometer (Coulter Electronics, Hialeah, FL). The MFI of fluorescence-positive cells was determined by computer analysis of the number of cells fluorescing in each channel on the Epics C. In some experiments, purified lung and blood T cells were stained and analyzed in the same manner. The machine was calibrated daily with standard beads (Fluospheres, Epics Division of Coulter Immunology, Hialeah, FL). All samples were analyzed at the same laser light output and photomultiplier gain settings.

In two-color analysis, 50 μL of heparinized blood or BAC suspension was placed into 12 × 75-mm tubes (Falcon 2052, Falcon Plastics, Oxnard, CA). Fifty microliters of FITC-conjugated and PE-conjugated monoclonal antibodies appropriately diluted with PBS/azide was then added simultaneously to the appropriate tubes. The samples were mixed and incubated for 30 min at 4°C in the dark. After hemolysis with the lysing solution, the samples were washed twice with PBS/azide, resuspended in 500 μL of PBS/azide and immediately analyzed on a Facscan flow cytometer (Becton Dickinson Immucytometry Systems, Mountain View, CA). Data from 10,000 cells were collected for each sample.

Statistical Analysis

All data were expressed as mean ± SD. The unpaired t test was used to assess significant differences between the MFI of lung lymphocytes and that of blood lymphocytes in each study group. The significance of differences among the six study groups was evaluated using one-way analysis of variance. Correlation coefficients were determined by the linear regression method of Pearson. For all comparisons, a p value of less than 0.05 was considered to be significant.

RESULTS

Expression of CD3

Figure 1 shows a representative overlay histogram of lung and blood lymphocytes stained with anti-CD3 monoclonal antibody in a patient with pulmonary sarcoidosis. Although the proportion of CD3+ cells in lung lymphocytes was higher than that in blood, their MFI was apparently reduced as compared with that in blood. Similar results were obtained in all study subjects. Thus, the MFI of CD3 on lung lymphocytes was significantly decreased compared with that on blood lymphocytes in all study groups (Fig 2). However, there were no significant differences in the MFI of CD3 on lung lymphocytes among the six study groups.

Since the modulation of CD3/TCR complex on lung T cells, as demonstrated by the reduced CD3 intensity, may be related to the mechanism by which T cells accumulate at the site of disease in the lung, we next examined the relationship between the MFI of CD3 on lung lymphocytes and the proportion of BALF lymphocytes. When all studies groups were combined, a weak but significant positive correlation was found between these parameters (r = 0.261, p < 0.05) (data not shown). A significant positive correlation was also found between the CD3 intensity on lung lymphocytes and the proportion of CD3+ cells in lung lymphocytes in overall study subjects (r = 0.521, p < 0.001) (Fig 3).

Because CD3 antigen is expressed on both CD4+ and CD8+ cells, it would be interesting to know in which subset of lung T cells CD3 molecules are more modulated. Two-color analysis in the limited number of subjects revealed that the amount of CD3 antigen on CD4+ lung lymphocytes was approximately equivalent to the amount present on CD8+ lung lymphocytes (data not shown).

Expression of CD4

Results concerning the expression of CD4 on lung lymphocytes were similar to those for CD3. Thus, the
MFI of CD4 on lung lymphocytes was significantly decreased compared with that on blood lymphocytes in most study groups (Fig 4). There were no significant differences in the MFI of CD4 on lung lymphocytes among the six study groups. To assess whether CD4 molecules are comodulated with CD3 molecules, the relationship between CD4 and CD3 intensities was determined in overall study subjects. The MFI of CD4 on lung lymphocytes was closely correlated with that of CD3 on lung lymphocytes \( (r = 0.848, p < 0.0001) \) (data not shown). A quite similar correlation was found in patients with pulmonary sarcoidosis \( (r = 0.957, p < 0.001) \) (data not shown).

**Expression of CD8**

Results regarding the expression of CD8 were different from those of CD3 and CD4. The MFI of CD8 on CD8\(^+\) lung lymphocytes did not differ significantly from that on CD8\(^+\) blood lymphocytes in normal subjects, patients with IPF, patients with CVD, patients with other pulmonary diseases, and patients with extrapulmonary diseases (Fig 5). However, the CD8 antigen on lung lymphocytes was expressed in a significantly higher density than that expressed on blood lymphocytes in patients with pulmonary sarcoidosis \( (p < 0.001) \). As can be seen in Figure 5, this was due to a decrease in the MFI of CD8 on blood lymphocytes in this disease, rather than to an increase on lung lymphocytes. In fact, the MFI of CD8 on blood lymphocytes in patients with pulmonary sarcoidosis was significantly lower than that in normal subjects \( (p < 0.01) \).

In two-color analysis, the examination of a two-dimensional contour plot of blood lymphocytes in a patient with pulmonary sarcoidosis revealed that CD8\(^+\) cell population was composed of bright CD8\(^+\), CD3\(^+\) cells and dim CD8\(^+\), CD3\(^-\) cells (Fig 6), the latter possibly representing natural killer cells. To determine the precise amount of the CD8 antigen expressed on CD3\(^+\) lymphocytes, bright CD8\(^+\), CD3\(^+\)
cells were gated and then analyzed for the MFI of CD8 in four patients with pulmonary sarcoidosis (Table 3). Results indicated that the CD8 antigen on this subset of blood lymphocytes was expressed in a density approximately equal to that on lung lymphocytes. Thus, there seemed to be no significant difference in CD8 antigen expression between lung and blood CD3+ lymphocytes in pulmonary sarcoidosis.

**Figure 4.** The MFI of CD4 on lung (stippled bar) and blood (open bar) CD4+ lymphocytes. *p<0.001; †p<0.01; ‡p<0.05 compared with blood lymphocytes in the same study group.

**Figure 5.** The MFI of CD8 on lung (stippled bar) and blood (open bar) CD8+ lymphocytes. *p<0.001 compared with blood lymphocytes in the same study group; †p<0.01 compared with blood lymphocytes in normal subjects.
To rule out several possible explanations for the difference in the expression of CD3 and CD4 antigens between lung and blood lymphocytes, such as nonspecific adsorption of antibodies on AM or other cell types in BALF and the effects of heparin in whole blood samples, lung and blood T cells were isolated by rosetting BAC or blood mononuclear cells with neuraminidase-treated sheep red blood cells and stained with anti-CD3 or anti-CD4 antibody. Similar down-regulation of the expression of these antigens on lung T cells was observed in all three patients with pulmonary sarcoidosis examined (data not shown).

**Discussion**

Our study demonstrated for the first time that the CD3 antigen on lung lymphocytes is expressed in lower density than that expressed on blood lymphocytes. This phenomenon was observed in all normal subjects and all patients with various pulmonary and extrapulmonary disorders in the present study. Since CD3 is known to be noncovalently linked to TCR,21 and TCR is comodulated with CD3,22 our observation provides indirect evidence of the modulation of TCR. Actually, we already have confirmed the modulation of TCR by the use of an antibody that directly recognizes the framework of TCR composed of α and β subunits in some of the study subjects (unpublished observation).

The simplest explanation for the modulation of CD3 on lung T cells in a wide variety of pulmonary disorders is that there is persistent antigenic stimulation of lung T cells via CD3/TCR complex regardless of the types of inflammatory process of the lung. Even in diseases in which specific antigens have not been identified, antigenic stimulation is most likely to be invariably present in alveolar spaces because of the contaminants borne in inhaled air during the normal process of respiration. In addition, AM and dendritic cells have been shown to have the capacity to present antigens to T cells.18,13,23 Therefore, it is possible that these antigen-presenting cells in the lung process and present antigens to lung T cells that are in close proximity to the antigen-presenting cells, thereby causing the modulation of the accessory molecules.

However, several observations in the present study seem to be inconsistent with the concept delineated above. There was a positive correlation between the MFI of CD3 on lung lymphocytes and the proportion of BALF lymphocytes, when all study subjects are combined. In addition, a significant positive correlation was found between the MFI of CD3 on lung lymphocytes and the proportion of CD3+ cells in BALF lymphocytes. Since these parameters are thought to reflect to some extent the local immune state of the lung, these facts mean that CD3 molecules on lung lymphocytes in subjects having high-intensity immune reactions undergo weak modulation, and seem to argue against the notion that the modulation of CD3 on lung lymphocytes results from an active antigen-presenting process between lung T cells and AM. It is also unlikely that antigens do exist in the local milieu in all patients with various extrapulmonary diseases, in which the modulation of CD3 on lung lymphocytes also was observed. Further studies are needed to elucidate the exact mechanism and the significance of the reduced expression of CD3 on lung T cells.

Regardless of the mechanism of the modulation of CD3 on lung T cells, our observations could partly account for the known hyporesponsiveness of lung T cells. Lecossier and his associates24 have reported that lung T cells are refractory to various proliferative signals. Consistent with this finding is our observation that the amount of interleukin-2 produced by phyto-

**Table 3—MFI of CD8 Antigen on CD3+ Cells Determined by Two-Color Analysis**

<table>
<thead>
<tr>
<th>Patients with Sarcoidosis</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8 intensity on lung CD8+ cells</td>
<td>126</td>
<td>135</td>
<td>121</td>
<td>130</td>
</tr>
<tr>
<td>CD8 intensity on blood CD3+ cells</td>
<td>134</td>
<td>134</td>
<td>124</td>
<td>125</td>
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</tbody>
</table>

*Data are expressed in channel numbers.
hemagglutinin-stimulated lung T cells is significantly decreased compared with that produced by blood T cells in patients with pulmonary sarcoidosis. Since it has been shown that the down-regulation of the cell surface CD3/TCR complex is correlated with the inhibition of antigen recognition and related immune responses to antigens or mitogens, our observations suggest that the modulation of CD3/TCR is involved in the hyporesponsiveness of lung T cells. Such down-regulation would be of physiologic relevance to the termination of the antigen-induced activation of lung T cells. In this context, it should be noted that AM have a limited capacity to secrete interleukin-1 compared with blood monocytes. Furthermore, they are less efficient antigen-presenting cells than blood monocytes. Thus, our observations seem to add another aspect of the mechanism whereby the local pulmonary milieu maintains the autoregulation of immune responses.

Although the exact roles of CD4 and CD8 molecules in T cell activation are presently a matter of controversy, it has been assumed that these molecules serve as associative recognition molecules that may bind to the nonpolymorphic parts of class 2 or 1 HLA antigen. In this connection, CD4 and CD8 are co-modulated by CD3 with antigenic or mitogenic stimulation. The present study showed that CD4 on lung lymphocytes was expressed in lower density than that on blood lymphocytes in most study groups. Furthermore, the MFI of CD4 on lung lymphocytes was closely correlated with that of CD3, thus providing convincing evidence for the co-modulation of CD4 and CD3. These results indicate that the general principle regarding antigen recognition via CD3/TCR complex holds good for CD4⁺ lung lymphocytes.

In contrast, normal subjects and patients with various pulmonary and extrapulmonary diseases other than pulmonary sarcoidosis had levels of CD8 antigen expression on lung lymphocytes similar to those on blood lymphocytes. Although the MFI of CD8 on lung lymphocytes was unexpectedly higher than that on blood lymphocytes in patients with pulmonary sarcoidosis, two-color immunofluorescence analysis revealed that this was merely a relative increase in CD8 antigen expression, which resulted from the presence of a considerable number of dull CD8⁺ lymphocytes in blood. Therefore, we conclude that, in general, CD8 molecules on lung lymphocytes do not undergo modulation.

The reason why CD8 on lung lymphocytes does not undergo modulation despite the modulation of CD3 on CD8⁺ lung lymphocytes is not sufficiently clear. Antigen recognition by lung CD8⁺ cells may be mediated by a different mechanism that has yet to be determined. Further studies are required to elucidate the events involved in the activation of lung CD8⁺ lymphocytes via CD3/TCR complex.

In summary, we have found the down-regulation of the expression of CD3 and CD4 antigen on lung T cells. This observation could represent indirect evidence for the modulation of T cell antigen receptors. Although its exact in vivo significance needs to be determined, it may account, at least in part, for the hyporesponsiveness of lung T cells.

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REFERENCES
5 Takada S, Engleman EG. Evidence for an association between CD8 molecules and the T cell receptor complex on cytotoxic T cells. J Immunol 1987; 139:3231-35
6 Anderson P, Blue ML, Schlossman SF. Comodulation of CD3 and CD4: evidence for a specific association between CD4 and approximately 5% of the CD3 T cell receptor complexes on helper T lymphocytes. J Immunol 1988; 140:1732-37
10 Yachie A, Hernandez D, Blaese RM. T3-T cell receptor (T) complex-independent activation of T cells by wheat germ agglutinin. J Immunol 1987; 138:2843-47
15 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American rheumatism association 1987