Systemic immune markers characterizing early stages of rheumatoid arthritis
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Chapter 7

Characteristics and potential involvement of CD70+ T-cells in seropositive arthralgia and in rheumatoid arthritis patients

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Work in progress
Abstract

Objectives
T-cells expressing CD70 are expanded in autoimmune diseases, including rheumatoid arthritis (RA). Upregulation of CD70 by CD4+ T-cells has been associated with a senescent phenotype on the one hand, while, on the other hand, CD70 has been reported as a specific marker for recently activated Th1 cells. We assessed if numbers of CD70+ T-cells (CD4+ and CD8+) were modulated by age, and characterized their phenotype and function by analysis of markers associated with activation, differentiation, cytokine production, proliferation and cellular senescence. Next, we assessed whether CD70+ T-cells are modulated in different phases of RA development.

Material and methods
Peripheral blood mononuclear cells (PBMC) were obtained from healthy controls (HC, n=19); seropositive arthralgia patients (SAP, n=28), representing subjects at risk of RA; newly diagnosed RA (n=23) and long-standing, treated RA patients (n=6). Also, synovial fluid mononuclear cells (SFMC) from late-stage RA were used. To characterize CD70+ T-cells, flow cytometric analysis using antibodies against CD45RO, CD27, CD28, CD69, HLA-DR, Ki-67, p16ink4a, T-bet, RORγt was performed. To investigate differences between T-cells from HC, SAP and early RA patients in their ability to regulate CD70 expression, PBMC were stimulated with PHA and cultured in vitro.

Results
Numbers of circulating CD70+ T-cells (CD4+ and CD8+) showed a strong positive correlation with age (p<0.0001). CD4+CD70+ T-cells were characterized by an increased expression of CD45RO, CD69, HLA-DR, Ki-67, p16ink4a and T-bet when compared to their CD70- counterparts. In RA, CD70+ T-cells were significantly increased at the level of the joint compared to the periphery. Frequencies of circulating CD70+ T-cells were not increased in PB of SAP and RA patients compared to HC. However, after in vitro stimulation, CD4+ T-cells from SAP and early RA showed an impaired ability to downregulate CD70 to baseline levels.

Conclusions
CD70+ T-cells (CD4+ and CD8+) increase with age and represent actively proliferating Th1-skewed effector cells rather than senescent T-cells. Accumulation of CD70+ T-cells at the level of the joint may suggest a role for these cells in augmentation of the local inflammatory process in RA. A delayed downregulation of CD70 by T-cells from SAP and RA may reduce the thresholds for activation of these cells and thereby contribute to the development of RA.
1. Introduction

Ample evidence suggests a central role of T lymphocytes in rheumatoid arthritis (RA) pathology (1, 2). The balance between T-cell activation and tolerance, crucial for immune homeostasis, is disrupted in RA and results in the dominance of a chronic immune effector response. The formation of such a response is dependent on the recognition of MHC-peptide complexes via specific T-cell receptor (TCR), as well as costimulatory signals derived from the same antigen presenting cells (APC). The most extensively studied costimulatory molecules, belonging to the B7/CD28 and TNF/TNFR families, include CD28 and CD27, respectively. Interaction between CD70 and its receptor CD27 is thought to be involved in the control of the tolerance-autoimmunity balance (3-5). Initiation of CD27-CD70 signaling is primarily regulated by the restricted expression of CD70. In contrast to CD27, constitutively present on the surface of T-cells, memory B-cells and NK-cells (6-8); CD70 is only transiently expressed on activated T-cells, B-cells, and dendritic cells (DCs) (9, 10).

Several studies demonstrated the involvement of excessive CD27-CD70 triggering in the pathogenesis of chronic infectious and autoimmune disorders. Increased numbers of CD70+ T-cells in systemic lupus erythematosus (SLE) have been implicated in the increased B-cell activation and antibody production (8, 11, 12). Similarly, blocking of the CD27-CD70 pathway in a mouse model of collagen-induced arthritis (CIA) led to a reduction of systemic autoantibody levels as well as decreased inflammation and joint destruction (13). Overexpression of CD70 on T-cells from rheumatoid arthritis (RA) patients has been demonstrated to provide a bystander co-stimulatory signal, derived not from APC, but third-party T-cells. This alteration, attributed to the defective T-cell ability to downregulate CD70 after stimulation, has been suggested to contribute to enhanced reactivity against low-affinity self-antigens (14).

Previously, CD70+ T-cells in RA were found to display a senescent phenotype (defined by their lack of CD28 expression) (14, 15). Accumulation of CD70+ T-cells in SLE has been associated with specific aspects of immune aging, i.e. the age-dependent decrease of DNA methylotransferase, involved in the suppression of CD70 gene transcription (5). We and others have demonstrated that CD70+ cells displayed an effector memory phenotype and an increased expansion potential (12, 16-19).

In this study, we first assessed if CD70+ T cells were modulated by age. We next characterized CD70+ T-cells in more detail by analysis of markers associated with activation, differentiation, cytokine production, proliferation and cellular senescence. Next, we assessed whether CD70+ T-cells home to the joint in late-stage RA. In order to investigate a role of CD70+ T-cells in RA development, the number of circulating CD70+ T-cells and the ability to regulate CD70 expression following in vitro stimulation were analyzed using peripheral blood mononuclear cells from seropositive arthralgia patients (SAP), who are at risk of developing RA (20-22) and from newly diagnosed RA patients.
2. Material and methods

2.1. Study participants

Venous blood collected in lithium heparin tubes was obtained from 19 healthy volunteers, 28 seropositive arthralgia patients (SAP), 23 recently diagnosed non-treated RA and 6 treated RA patients. Synovial fluid samples were collected from late-stage RA patients. Early RA patients had their blood drawn at time of diagnosis, before start of treatment with disease modifying anti-rheumatic drugs (DMARDs). Both SAP and early RA were treated with non-steroidal anti-inflammatory drugs (NSAIDs) only. Late-stage RA patients received methotrexate (3/6), prednisone (1/6), TNF-α inhibitors (3/6) and rituximab (1/6). All RA patients fulfilled the 1987 or 2010 American College of Rheumatology (ACR) classification criteria for RA. Absence of arthritis in SAP was confirmed by physical examination of 44 joints by a trained senior rheumatologist (EB). All participants gave their informed consent, and the study was approved by the local medical ethics committee (UMC Groningen, The Netherlands). Demographical and clinical characteristics of the included subjects are shown in Table 1.

Table 1. Demographical and clinical characteristics of the subjects included in the study.

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>SAP</th>
<th>Early RA</th>
<th>RA</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>19</td>
<td>28</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>Age [yrs]; mean (SD)</td>
<td>53.0 (7.5)</td>
<td>51.0 (14.6)</td>
<td>53.8 (12.6)</td>
<td>53.3 (5.9)</td>
</tr>
<tr>
<td>Gender; % female (n)</td>
<td>52.6 (10)</td>
<td>64.3 (18)</td>
<td>56.5 (13)</td>
<td>50.0 (3)</td>
</tr>
<tr>
<td>ACPA positive; % (n)</td>
<td>nd</td>
<td>92.9 (26)</td>
<td>73.9 (17)</td>
<td>100 (6)</td>
</tr>
<tr>
<td>RF positive; % (n)</td>
<td>10.5 (2)</td>
<td>85.7 (24)</td>
<td>73.9 (17)</td>
<td>66.7 (4)</td>
</tr>
<tr>
<td>CRP [mg/l]; median (range)</td>
<td>nd</td>
<td>5.0 (5.0-29.0)</td>
<td>12.0 (5.0-57.0)</td>
<td>5.0 (5.0-34.0)</td>
</tr>
<tr>
<td>ESR [mm/h]; median (range)</td>
<td>nd</td>
<td>11.5 (2.0-69.0)</td>
<td>22.0 (2.0-80.0)</td>
<td>15.0 (6.0-29.0)</td>
</tr>
<tr>
<td>TJC [n]; median (range)</td>
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<td>7.0 (0.0-23.0)</td>
<td>2.0 (0.0-4.0)</td>
</tr>
<tr>
<td>SJC [n]; median (range)</td>
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<td>0.0 (0.0-7.0)</td>
<td>6.0 (0.0-9.0)</td>
<td>2.0 (1.0-6.0)</td>
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<td>na</td>
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<td>3.3 (1.1)</td>
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<td>Erosions; % (n)</td>
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<td>na</td>
<td>4.3 (1)</td>
<td>66.7 (4)</td>
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</table>

HC = healthy controls; SAP = seropositive arthralgia patients; RA = rheumatoid arthritis; ACPA = anti-cyclic citrullinated proteins antibodies; RF = rheumatoid factor; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; TJC = tender joint count; SJC = swollen joint count; DAS28 = disease activity score 28; nd = not done; na = not applicable

2.2. Mononuclear cell isolation and staining

Mononuclear cells from peripheral blood (PBMC) and synovial fluid (SFMC) were isolated by Lymphoprep™ (Axis-Shield, Oslo, Norway) density gradient centrifugation. Isolated cells were resuspended in RPMI-1640 containing 10% fetal bovine serum (FBS; Lonza, Breda, the Netherlands) and 10% dimethyl sulfoxide (DMSO) on ice. The cell suspension was transferred in cryovials to Nalgene™ Cryo 1°C freezing containers (Thermo Scientific, Langenselbold, Germany) and placed in -80°C
overnight, followed by storage in liquid nitrogen until analysis. Thawed PBMC and SFMC were resuspended in PBS with 1% bovine serum albumin (BSA; Sigma-Aldrich, Zwijndrecht, The Netherlands) at a concentration 10^6 cells/100 µL and stained with the following anti-human antibodies: CD70 FITC, CD70 PE, CD45RO PE-Cy7, HLA-DR AF700, CD8 PerCP (BD Biosciences, Breda, the Netherlands), CD3 eF605NC, CD4 eF450, CD4 APC-eFluor780, CD27 PerCP-eFluor780, CD27 AF700 (eBioscience, Vienna, Austria), CD19 APC-Cy7, CD28 APC, CD38 AF700, CD69 PE-Cy7 (BioLegend, San Diego, CA, USA). For the intracellular markers detection, cells were fixed and permeabilized with the Foxp3/Transcription Factor Staining Buffer Set (eBioscience). Antibodies against human Ki-67 PerCP-Cy5.5, p16ink4a PE (BD Biosciences) or T-bet PerCP-Cy5.5 and RORγt APC (eBioscience) were added to the cell suspensions in the permeabilization buffer (eBioscience) and incubated for 30 min at RT. Following washing with the permeabilization buffer (eBioscience), cells were analyzed using LSR II flow cytometer (BD Biosciences). Data analysis was performed with Kaluza® analysis software (Beckman Coulter, Woerden, The Netherlands).

2.3. PBMC in vitro stimulation and staining
Following thawing, PBMC were resuspended in RPMI-1640 with 10% FBS and 0.6% gentamycin (Life Technologies, Bleiswijk, The Netherlands), at a final concentration 10^6 cells/mL. Phytohaemagglutinin (PHA) was added at a final concentration 5 µg/mL. At day 4 and day 8 after start of the in vitro culture, cells were collected from wells and washed three times with medium. Next, cells were resuspended in RPMI-1640 with 10% FBS and 0.6% gentamycin at a final concentration 10^6 cells/mL. In vitro culture was continued in the presence of human recombinant IL-2 (Peprotech, London, UK) in a final concentration 100 U/mL. At day 13 after start of the in vitro culture, cells were washed and resuspended in fresh medium as previously and the culture was continued for the next 6 days without exogenous IL-2. Aliquots of PBMC collected at day 0, 4, 8, 13 and 18 during in vitro culture were used for analysis of the expression of surface and intracellular markers according to the procedure described above.

2.4. Statistical analysis
Statistical analysis was performed with GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). Correlations between the continuous variables were analyzed with Spearman’s rank correlation coefficient. Paired samples analysis was performed with Wilcoxon signed rank test. Non-normally distributed independent samples were compared using Mann-Whitney 2-tailed test. P<0.05 was considered statistically significant.
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3. Results

3.1. Numbers of CD70+ T-cells increase with age
Both CD4+CD70+ and CD8+CD70+ T-cells correlated positively with age. This was observed for both the frequency and the absolute numbers of CD4+CD70+ (p<0.0001, r=0.55 and p=0.004, r=0.41, respectively) and CD8+CD70+ T-cells (p<0.0001, r=0.53 and p=0.017, r=0.34, respectively; Fig.1A,B).

3.2. CD70+ T-cells differ from CD70- T-cells in their phenotypes
We aimed to elucidate whether the observed increase of CD70+ T-cells with age is due to a gradual increase of senescent T-cells or due to expansion of the activated effector memory T-cell pool. CD70+ T-cells from healthy controls were analyzed for the expression of markers associated with a more differentiated phenotype such as surface CD28, CD27, CD45RO and expression of the intracellular senescence-associated p16ink4a. Also, markers associated with the activated cellular phenotype such as CD69, HLA-DR and the proliferation marker Ki-67, were assessed. To assess the putative function of age-associated CD70+ T-cells, we analyzed the expression of the transcription factors T-bet and RORγt, activators of IFN-γ and IL-17, respectively.

3.2.1. Markers of T-cell differentiation and senescence
Nearly all CD4+CD70+ T-cells expressed CD45RO, CD27 and CD28, indicating their memory, but not terminally differentiated phenotype (Fig. 1C). In contrast, CD4+CD70- T-cells showed significantly lower CD45RO expression (median 44.5% in CD4+CD70- vs. 89.8% in CD4+CD70+) and significantly higher CD27 expression (median 97.0% in CD4+CD70- vs. 91.1% in CD4+CD70+) suggesting a less differentiated phenotype. The percentages of CD28+ cells within both populations were similar. A small proportion (median 1.5%) of CD4+CD70+ T-cells expressed the senescence- associated p16ink4a which was found to be somewhat higher compared to CD4+CD70- T-cells (median 0.6%).

3.2.2. Markers of T-cell activation and proliferation
CD4+CD70+ T-cells were characterized by a significantly higher expression of the activation markers: CD69 (median 7.1% in CD4+CD70+ vs. 3.1% in CD4+CD70-) and HLA-DR (median 12.2% in CD4+CD70+ vs. 0.8% in CD4+CD70-).

The most pronounced difference between CD4+CD70+ and CD4+CD70- T-cells was the expression of Ki-67. Median 18.8% of CD4+CD70+ cells were Ki-67+ while only 1.4% of CD4+CD70- cells expressed Ki-67, suggesting that CD70+ T-cells in vivo show higher proliferation rates.

3.2.3. Transcription factors T-bet and RORγt
T-bet and RORγt were expressed by a minor fraction of both CD70+ and CD70- T-cells. However, CD4+CD70+ T-cells expressed T-bet at a significantly higher level than CD70- T-cells (median 1.3% in CD4+CD70+ vs. 0.3% in CD4+CD70-), while the expression of RORγt within both subsets was found to be similarly low (median 0.7% in CD4+CD70+ vs. 0.4% in CD4+CD70-). Similar observations for these markers were made for CD8+CD70+ and CD8+CD70- T-cells (Fig.1D).
Figure 1. CD70+ T-cells (both CD4 and CD8) increase with age and show increased expression of markers associated with activation and proliferation.

A) CD4+CD70+ and B) CD8+CD70+ T-cells from 49 donors, including 10 HC, 28 SAP and 11 early RA were correlated with age (Spearman’s rank correlation). Directly ex vivo isolated CD70+ and CD70- T-cells from the C) CD4+ and B) CD8+ T cell population obtained from HC were compared for surface expression of CD28, CD27, CD45RO, CD69, HLA-DR and intracellular expression of Ki-67, p16ink4a, T-bet and RORγt (Wilcoxon sign rank test). Bars and whiskers represent median and interquartile range. * indicates p<0.05.

3.3. Frequencies of peripheral blood CD4+CD70+ and CD8+CD70+ T-cells are not different in HC, SAP and RA patients

Increased frequencies of CD70+ T-cells have been described in various autoinflammatory conditions, including RA (14, 19). Within our cohorts of SAP, recently diagnosed RA and long-standing RA patients on treatment, the observed frequencies of CD4+CD70+ and CD8+CD70+ T-cells were not different from age-matched HC (Fig2A). Also, the phenotype of these cells did not differ from HCs (data not shown). Frequencies of CD70+ T-cells in SAP and RA did not correlate with clinical characteristics, such as CRP,
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ESR, DAS28, TJC, SJC or RF level (data not shown). A weak positive correlation between the levels of ACPA and CD4+CD70+ T-cells was found (p=0.019, r=0.38; Fig.2B), in contrast to CD8+CD70+ T-cells (p=0.51, r=0.11). Interaction between CD70+ Th cells and CD27+ memory B-cells has been reported to induce plasma cell differentiation and subsequent immunoglobulin production (7, 8, 23, 24). We analyzed the correlation between the frequency of CD4+CD70+ T-cells and circulating plasma cells defined as CD27<sup>high</sup>CD38+. No statistically significant correlation between these subsets was observed. Neither did the frequency of CD4+CD70+ T-cells correlate with the number of naïve (CD27-CD38+), transitional (CD27-CD38+) or memory B-cells (CD27+) (data not shown).

3.4. CD70+ T-cells are significantly increased in RA synovial fluid

To investigate whether CD70+ T-cells may play a role at the site of local inflammation, their frequencies were analyzed in paired samples of PB and SF obtained from 6 long-standing RA patients. Both CD4+ and CD8+ T-cells expressing CD70 were found at significantly higher frequencies in SF (median 12.1% and 16.9%, respectively) compared to PB (median 2.0% for both CD4+ and CD8+) (Fig. 2C,D).

Figure 2. CD70+ T-cells accumulate in synovial fluid in late-stage RA.

Frequencies of CD4+CD70+ and CD8+CD70+ T-cells were assessed in PB of 10 HC, 28 SAP, 11 early RA and 6 late-stage RA patients (Mann-Whitney test). B) Correlation between the frequency of CD4+CD70+ T-cells and ACPA titers from 28 SAP and 11 early RA (Spearman’s rank correlation). Frequencies of C) CD4+CD70+ and D) CD8+CD70+ T-cells in PB and SF of late-stage RA patients (n=6). Dot plots from 1 representative patient are shown. Graphs depict the pooled data from all late-stage RA patients (Wilcoxon sign rank test). * indicates p<0.05.
3.5. CD70 is transiently upregulated on the vast majority of T-cells following stimulation

Following characterization of ex vivo-derived CD70+ T-cells, we aimed to assess the expression kinetics of CD70 induction and the relationship between expression of CD70 and CD28, p16ink4a, CD69, HLA-DR or Ki-67. For that purpose, we performed an 18-day in vitro culture of PBMC. After 4 days in the presence of PHA, most of CD4+ T cells (median ~93%) were found to express CD70. Further culture in PHA-free medium led to a gradual downregulation of CD70 expression. Five (D8), ten (D13) and fifteen (D18) days after mitogen withdrawal, ~38%, ~13% and ~3% of CD4+ T-cells expressed CD70, respectively (Fig.3A). A similar induction and downmodulation of CD70 expression was seen in CD8+ T cells (data not shown). Percentages of CD70 expressing cells were higher than seen in CD4+ T-cells. At day 4, 8, 13 and 18 after start of culture, the median frequency of CD8+CD70+ T-cells was ~98%, ~54%, ~18% and ~3%, respectively (data not shown).

CD4+ T-cells expressing CD70 at day 4 were also positive for CD28. Since nearly all CD4+ T lymphocytes expressed CD28 at baseline, no further increase in the percentage of CD28 expressing cells was observed (Fig.3A). Despite the fact that CD70 upregulation was activation-dependent, only a fraction of CD70+ T-cells co-expressed other markers of activation, such as CD69 and HLA-DR (Fig. 3B,C). The frequency of CD70- T-cells expressing CD69 or HLA-DR during culture remained at a low level of ≤10%.

We observed that the induction of CD70 expression was associated with higher expression of Ki-67, indicative of cellular proliferation. Yet, not all proliferating cells showed CD70 expression. The percentage of CD70-Ki-67+ cells was found to be ~26% within the CD4+ T-cell population (Fig.3D). As demonstrated for CD69 and HLA-DR, also p16ink4a was found to be co-expressed by CD70+ cells following stimulation. The frequency of CD4+ expressing p16ink4a, but negative for CD70 was <4% (Fig. 3E). Similar expression kinetics were observed for CD8+ T-cells (data not shown).

In conclusion, in vitro activation of T-cells leads to co-expression of CD70 primarily with markers of T-cell activation and proliferation.

3.6. In vitro stimulated T-cells from SAP and early RA show delayed CD70 downregulation

We next analyzed the dynamics of CD70 expression in the patient groups. During in vitro stimulation and subsequent gradual return to the resting phase, CD4+ T-cells from SAP and RA showed a decreased ability to downregulate CD70 compared to HC T-cells. Ten days after stimulus withdrawal (D13), a significantly higher frequency of SAP (18.4%, p=0.009) and RA T-cells (18.9%, p=0.037) expressed CD70 compared to HC (12.7%). Five days later (D18) SAP and RA CD4+ T-cells had downregulated CD70 expression to the level observed with CD4+ T-cells from HC (Fig.4A). CD8+ T-cells from SAP and RA showed a trend for a similarly defective downregulation of CD70, when compared to HC. However, the differences between SAP or RA and HC at day 13 (p=0.094, p=0.073, respectively) or at day 18 (p=0.054, p=0.097, respectively) did not reach statistical significance (Fig.4B).
Figure 3. Dynamics of CD70 expression and other markers of T cell activation and proliferation upon in vitro T-cell activation.

A-C) PBMC obtained from 7 HC were stimulated with PHA for 4 days followed by washing and culture in the presence of IL-2 alone for the next 10 days. After that, PBMC were maintained in culture with medium alone for the next 5 days. Co-expression of CD70 with A) CD28, B) CD69, C) HLA-DR was assessed at day 0, 4, 8, 13 and 18 of the culture. D), E) PBMC obtained from 6 HC were stimulated in the same way as described above. Co-expression of CD70 with D) Ki-67 or E) p16ink4a was assessed at day 0, 4 and 18 of in vitro culture. Representative dot plots from 1 HC are shown. Mean frequencies from the 4 quadrants within the dot plots are visualized by pie charts (mean of n =7 or n =6 HC data).
Figure 4. Delayed CD70 downregulation by T-cells from SAP and RA.

Analysis of stimulation-induced CD70 expression regulation within A) CD4+ and B) CD8+ T-cells in long-term in vitro culture. PBMC obtained from 7 HC, 8 SAP and 7 early RA patients were ex vivo stimulated with PHA and CD70 expression by CD4+ and CD8+ T-cells was analyzed by flow cytometry (Mann-Whitney test). Symbols in the graphs represent median with interquartile range. * indicates p<0.05.

4. Discussion

Expansion of T lymphocytes expressing CD70 has been reported in various autoimmune conditions, including SLE (11, 12), Sjögren’s syndrome (25), systemic sclerosis (26) and RA. In SLE, increased CD70 expression has been attributed to the age-related decline of T-cell DNA methyltransferase levels and subsequent hypomethylation of the CD70 promoter region (5, 15). In contrast, CD70 overexpression in RA has been attributed to the ill-defined defects in its downregulation (not associated with CD70 promoter hypomethylation) (14). In normal conditions, CD70 is transiently upregulated on activated T-lymphocytes, followed by a gradual downregulation (6, 10, 14, 16). In RA T-cells, reciprocal CD70 expression and acquisition of the senescent phenotype, manifested by CD28 loss, has been reported (14, 15). On the other hand, CD70+ T-cell expansion in SLE has been suggested to represent an activation-dependent rather than an age-dependent alteration (12). In our study, we did not observe increased frequencies of CD70+ T-cells in RA compared to age matched healthy controls. However, a significant positive correlation between the number of CD70+ T-cells and age prompted us to investigate the putative cause of the observed expansion. We aimed to establish whether CD70+ T-cells, increasing in elderly, represent aged T-cells bearing features of replicative senescence. Alternatively, age-associated systemic low grade inflammation (inflammaging) (27) may mediate bystander T-cell activation and accumulation of CD70+ effector T-cells.

Our data indicate that, in line with the second hypothesis, CD4+CD70+ T-cells show features of primed (CD45RO+, CD69+, HLA-DR+), actively proliferating (Ki-67+) cells, skewed toward the Th1 phenotype (T-bet+) when compared to CD4+CD70- T-cells. This is consistent with a study of Kawamura et al which demonstrated CD70 expression to be specific for Th1 but not Th2 cells (17).
Similar characteristics, including increased expression of T-bet within the CD8+CD70+ T-cell subset, suggest that CD70+ expression is associated with enhanced effector functions that could translate in vivo into increased IFN-γ production and cytotoxicity (28, 29) when compared to CD8+CD70- T-cells. Similar to CD70+ T-cells characterized directly following ex vivo isolation, T-cells with stimulation-induced de novo CD70 expression were also positive for CD28 and Ki-67.

Our data confirm a previous observation by Lee et al on defective downregulation of stimulation-induced CD70 expression in RA (14). We here report that stimulation-induced CD70 downregulation is similarly delayed in SAP. This indicates a possible role for prolonged CD70 expression in RA development, as ~30% SAP has been shown to develop RA (20-22). Overexpression of CD70 on T-cells has been suggested to contribute to the induction of autoreactivity by virtue of lowering the activation thresholds of low-affinity T-cells (including those specific for self-antigens). It may also contribute to RA pathology by augmenting autoantibody production, as frequencies of CD4+CD70+ T-cells were found to correlate with ACPA titers. Indeed, CD27-CD70 interaction was shown to be involved in Th-mediated B-cell differentiation, plasma cell generation and immunoglobulin expression (7, 8, 23, 24). However, similar frequencies of CD4+CD70+ T-cells in RA patients and healthy controls suggest that additional RA-related factors are involved in autoantibody induction. Results of the study by Sammicheli et al. imply a pivotal role of IL-7 in this process (18). IL-7 was shown to induce CD70 expression on CD4+ T-cells which led to the subsequent CD70-mediated B-cell activation and immunoglobulin production. Furthermore, IL-7 was shown to induce expression of B cell activating factor (BAFF) which, in concert with anti-IgM F(ab')2-fragments, has been demonstrated recently to stimulate ACPA production in vitro (30). Systemic levels of both IL-7 (31) and BAFF (32) have been shown to be markedly increased at the early stages of RA.

We confirmed accumulation of CD70+ T-cells in RA SF (33). CD70+ T cells may be involved in the recognition of (citrullinated) self or foreign peptides in the joints. IL-7, shown to induce CD70 expression, can also increase sensitivity of T-cells towards low-affinity self-antigens, including citrullinated peptides (34). We hypothesize that CD70+ T-cells preconditioned by the increased systemic IL-7, followed by triggering of TCR by self- peptide/MHC complexes in the local lymph node, would migrate preferentially towards the arthritic joint. Clearly, future studies are required to investigate this hypothesis.

In conclusion, our study confirms the notion that CD70+ T-cells represent activated, actively proliferating Th1-skewed effector cells rather than terminally differentiated T-cells. Further studies should elucidate the role of CD70+ T-cell accumulation in normal aging. In early stages of RA (SAP and recently diagnosed RA), numbers of circulating CD70+ T-cells were not different from age-matched healthy subjects. However, defective downregulation of CD70 expression following stimulation in SAP and RA, as well as positive correlation with ACPA levels were observed. Further studies are required to understand the implications of these alterations for RA development.
CD70+ T-cells in RA

References


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