Selective migration of memory T cells to the synovial tissue in arthritis (summary of two recent papers).

Introduction
Whether the chronic progression in rheumatoid arthritis (RA) is due to a continuing presence of foreign antigen(s) in the joint, or whether T cells are generated that cross react with joint auto antigens is not clear. In either case one might expect to find elevated reactivity of T cells to the antigen in synovial tissue (ST), compared with blood. With this study the potential role of pathogens as causative agents in rheumatoid arthritis (RA) was investigated by comparing proliferative capacity of ST and peripheral blood (PB) derived T cells to 18 viral, bacterial and protozoan antigens (1).

The migration of T cells with different antigen specificities to synovial tissue supernatant from RA patients was also investigated to see whether the migratory capacity of T cells is related to the antigen specificity of the T cell and whether the specificity of the T cell is reflected in chemokine receptor profile. Chemokines involved in migration were identified by blocking individual chemokines using monoclonal antibodies (2).

Methods
Depletion of naive T cells from PBMC
Naive T cells were depleted from PBMC (peripheral blood mononuclear cells) with 99% purity, using anti-CD45RA (HI100) (PharMingen, US) coated onto Dynabeads® Sheep anti-Mouse IgG (Dynal Biotech, Norway).

Briefly, prewashed Dynabeads® were incubated with 1 mg anti-CD45RA/1 x 10^7 Dynabeads at 4°C overnight, and washed with PBS/0.1% BSA. PBMC were resuspended in PBS/2% FBS and anti-CD45RA coated Dynabeads®/target cell followed by gentle rocking at 4°C for 30 min. The captured cells were separated with a Dynal MPC®-L magnet. The remaining cells were washed with PBS/0.1% BSA and resuspended in RPMI 1640/10% human serum.

T cell proliferation assay
1 x 10^6 PBMC or STMC (synovial tissue mononuclear cells) were incubated with antigen for 4 days. Over the last 18 hours they were pulsed with 0.5 μCl of tritiated thymidine (Amersham Biosciences, U.K.). The incorporated thymidine was determined with a beta counter. Stimulation Index (SI) was calculated. Antigens included: Protozoa: Acanthamoeba polyphaga (AP), Viruses: Herpes simplex virus (HSV), Cytomegalovirus (CMV), Varicella zoster (VZV), Mumps, Rubella, Rubeola, Bacteria: Shigella (S) flexneri, S. infantis, Helicobacter (H) pylori, Campylobacter (C) jejuni, Yersinia (Y) enterocolitica, Klebsiella (K) pneumoniae. Phytohemagglutinin was used as positive control (1).

T cell lines
PBMC depleted of naive T cells were initially stimulated with AP, HSV or C. jejuni. T cell lines from 5 patients were used after four cycles (2).

Chemokine receptor phenotyping of T cell lines and clones
Chemokine receptors were stained and analysed by flow cytometry. The antibodies used were anti-CCR1 (5504.111), anti-CCR2 (48607.121), anti-CCR5 (CTC8), anti-CCR6 (53103.111), anti-CXCR3 (49801.111), anti-CXCR4 (FAB170P) (all from R&D Systems, UK) and PE-conjugated anti-mouse IgG antibody. CCR7 was stained with anti-CCR7 (2H4), biotin-conjugated rat anti-mouse (R6-60.2) and streptavidin-PE conjugate (554061) as per the supplied protocol (PharMingen) (2).

Trans-migration assays
T cell migration and chemokine blocking experiments were done with a microchemotaxis chamber, (ChemoTx®;Neuroprobe,US). ST-derived supernatant, or supernatant treated overnight with titrated antibody concentrations of anti-MIP-1b (24006.111), anti-RANTES (MAB278), anti-MIP-3α (67310.111), anti-IP-10 (33036.211) (R&D Systems) or anti-MCP-1 (20520D) (PharMingen), or isotype matched controls, was used as a chemo-attractant (2).

<table>
<thead>
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<th>Antigen group</th>
<th>ST mean SI +/-SEM</th>
<th>PB mean SI +/-SEM</th>
<th>Difference between means (P value)</th>
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<tr>
<td>Virus</td>
<td>5.81 +/-0.51</td>
<td>9.37 +/-2.20</td>
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<td>Bacteria</td>
<td>3.56 +/-0.26</td>
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<td>Amoeba</td>
<td>1.03 +/-0.11</td>
<td>6.38 +/-0.32</td>
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* The difference is significant
Results and conclusion

A high proliferative capacity of all RA patients was derived and almost no proliferative response of the STMC from the same patients to the AP antigens was observed. All the other antigens gave proliferative responses in both compartments (Table 1) (1). PB derived T cell lines from RA patients, specific for HSV and C. jejuni also showed a significantly higher capacity to migrate towards chemokines produced by ST derived cells than AP specific T cell lines and clones (Fig. 1).

To reveal the molecular basis for the different homing capacities of T cells with different specificities, the chemokine receptor expression profile of the T cell lines was measured. This revealed that HSV and C. jejuni specific T cell lines strongly expressed CXCR3, CCR5 and CCR2 compared to AP specific T cell lines (Fig. 2).

Migration of two C. jejuni specific- and two HSV specific T cell lines were inhibited by an average of 60% when the ST derived supernatant was treated overnight with antibodies against RANTES or MCP-1 (Fig. 3). T cell migration was not significantly affected by addition of anti-iP-10 antibodies (data not shown) (2).

In conclusion, the data indicate that AP specific T cells have a reduced capacity to migrate into ST. Neutralisation of the chemokines RANTES or MCP-1 in the ST cell derived chemo-attractant profoundly reduced T cell migration, implicating that each chemokine and chemokine receptor serves a unique role in T cell migration. RANTES interactions and MCP-1 interactions may play significant roles in T cell migration to inflamed ST. The studies also indicate that pathogenic antigens are able to influence the chemokine receptor expression on antigen specific T cells during activation, and thus influence their migratory abilities. Further studies on the involvement of CCR5 and CCR2 in the migration of T cells to the synovium might result in new therapeutic approaches in RA (2).

References


Ordering information

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