Immune-inflammatory response in infected arthroplasties

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We studied the immunocytology of synovial fluid in purulent endoprosthetic infections using cell subtype-specific monoclonal antibodies in avidin-biotin-peroxidase complex staining. Two thirds of the monocytes were CD15-positive, whereas CD2-positive T lymphocytes only formed one third of all the mononuclear cells. The synovial fluid monocyte-activated T-cell ratio differed from findings in sterile inflammatory, reactive and rheumatoid arthritis.

Despite the recent thorough characterization of synovial cell immunocytology in inflammatory and reactive arthritides (Duclas et al. 1982, Duke et al. 1983, Nordström et al. 1985, Konttinen et al. 1986, Nilsson 1987), the possible use of this method in purulent endoprosthetic infection has not been determined. We report the composition of the mononuclear cells in synovial fluid in endoprosthetic infection using monoclonal hybridoma antibodies in avidin-biotin-peroxidase complex staining.

Patients and methods

Synovial fluid was obtained by arthrocentesis from 4 patients with an infected arthroplasty (three hips and one knee; Table 1). For comparison, knee exudate was obtained from 5 patients with rheumatoid arthritis and from 5 patients with HLA-B27-positive reactive arthritis in whom the triggering infection (*Ysersinia* in 2 patients, *Salmonella* in 2 patients, and *Chlamydia* in 1 patient) had been identified by microbial cultures and/or serologic tests.

Synovial-fluid mononuclear cells were isolated using Lymphoprep® (1.078 g/mL; Nyegard and Co. A/S, Oslo, Norway) density-gradient isolation. Cytocentrifuge slides were prepared from the washed cells. After air drying and acetone fixation (5 min at 4 °C), endogenous peroxidase was inhibited using 0.3 percent H_2O_2-PBS. For immunoperoxidase staining, we used avidin-biotin-peroxidase complex staining (Hsu et al. 1981). Briefly, specific identification was based on the use of the following monoclonal antibodies (IUIS-WHO Nomenclature Subcommittee 1984) used in 1:20–1:100 dilution: anti-CD2, anti-CD4, anti-CD8, anti-CD15, and anti-CD25, which identified total T cells, inducer/helper T cells, suppressor/cytotoxic T cells, monocytes, and interleukin-2 receptors, respectively. Biotinylated horse antimouse IgG in the second layer was diluted to 1:250 and followed by a further 30-min

Table 1. Clinical data of endoprosthesis infection patients studied

Patient	Sex	Age	Joint	Prosthesis	Time operation – infection	Microb	Treatment
1	F	78	Left knee	Guepar	12 days	*Staph. epid.*[a]	Oxacillin
2	F	73	Left knee	PCA	12 days	*Proteus Mirabilis Enterob. Cloacae*	Co-trimo-Xazole Cephradine
3	F	62	Left knee	Guepar	32 days	*Staph. epid.*[a]	Oxacillin
4	F	44	Right hip	Lord	12 months	*Staph. aureus*[a]	Oxacillin

[a] Penicillinase-producing strains.

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incubation at room temperature with avidin-biotin-peroxidase complexes. Immune-attached exogenous peroxidase was visualized using 3.3 diaminobentzidine tetrahydrochloride as a substrate and H_2O_2 as the electron donor. Cleared and dehydrated specimens were mounted in Histoclad and evaluated by light microscopy (Figure 1).

At least 200 cells were counted from each specimen using an oil immersion objective (1,000x) and a 10x 10-square ocular counting ridge. Results were expressed as a percentage, and the standard error of the mean was used to express dispersion. Differences between various groups were tested using Wilcoxon's two-sample, rank sum test. Sensitivity was calculated as true positive/(true positive + false negative) x 100 percent. Specificity was calculated as true negative/(true negative + false positive) x 100 percent. The predictive value of the positive test for purulent arthritis was calculated as true positives/apparent positives and the predictive value of the negative test as true/apparent negatives.

Results

In infection, CD15-positive monocytes were the predominant synovial-fluid mononuclear cells (Table 1), whereas T lymphocytes were the predominant cells in reactive and rheumatoid arthritis. Further, CD25-positive, activated, interleukin-2 receptors with T cells were especially sparse in infection and most frequent in reactive arthritis in the synovial fluid, which is known to be a site for an actively ongoing, local, cell-mediated immune response (Konttinen et al. 1986). Because of the monocyte-T lymphocyte difference, on the one hand, and the relative indolence of T cells in infection, on the other, the CD15/25 ratio was especially useful in distinguishing (Table 2) infection (58 ±20) from immune-inflammatory reactive arthritis (0.8 ± 0.3; $P < 0.05$) and rheumatoid arthritis (3.4 ±0.6; $P < 0.05$; Wilcoxon's two-sample rank sum test). If, for instance, 10 was taken as as a cutting-off point for the CD15/25 ratio, then, the specificity and sensitivity of the CD15/25 ratio for purulent arthritis against both reactive and rheumatoid arthritides were 100 percent. The predictive value of the positive test for purulent arthritis against both reactive arthritis and rheumatoid arthritis was 1.0, and the predictive value of the negative test for purulent arthritis against both reactive and rheumatoid arthritis was infinite.

Table 2. Comparison of various mononuclear cell differentiation and activation markers in purulent (Pu), reactive (Re), and rheumatoid (R) arthritis. Percentage *SE*

	CD2	CD4	CD8	CD15	CD25
Pu	34 *15*	21 *9*	13 *7*	64 *16*	1 *0.4*
Re	61 *11*	35 *11*	24 *7*	26 *11*	33 *4*
R	82 *4*	27 *6*	55 *5*	12 *3*	4 *1*
P-value[a]					
Pu vs Re	NS	NS	NS	NS	<0.05
Pu vs R	<0.05	NS	<0.05	<0.05	NS

[a] Wilcoxon's rank sum test.

Discussion

Purulent arthritis, in general, seems to be characterized, in contrast to sterile immune-inflammatory arthritides, by a rapid, but nonspecific, neutrophil-dominated inflammatory response. This is reflected in the synovial-fluid leukocyte and differential counts (Cohen and Goldenberg 1985), and this response is also used in the scanning of indium-111-labeled neutrophils (Syrjälä et al. 1987).

Despite the advances in monoclonal hybridoma-antibody production, in immunohistochemical staining techniques, and also in cytofluorographic analysers, this is the first report on immunocytology of endoprosthetic infections. Our study suggests that the mononuclear-cell response in infection is strikingly characterized by a monocyte-mediated, nonspecific, but rapid, inflammatory response.

In agreement with earlier reports, we found that the T lymphocyte is the predominant cell subset in immune-inflammatory reactive arthritis and rheumatoid arthritis (Duclas et al. 1982, Duke et al. 1983, Nordström et al. 1985, Nilsson 1987). This accords with the supposed role of the slow, but specific, lymphocyte-mediated autoimmune response of these diseases (Aho 1984, Zvaifler 1985). Further, our observation of the proportion of activated interleukin-2 receptor-positive T blasts in the various arthritides is a useful example of the value of assessment of the lymphocyte activation state. It appears that the proportion of activated T blasts is a better discriminator of various arthritides than the total T-cell counts, disregarding the activation state. The CD15/25 ratio, in particular, was useful in the classification of synovial-fluid samples into the broad categories of infectious and noninfectious conditions.

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