Originals

Interleukin-6 localisation in the synovial membrane in rheumatoid arthritis

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Summary. Polyclonal antibodies were raised in rabbits against Interleukin-6 (IL-6) by immunisation with a synthetic peptide of identical sequence to the amino terminal 12 amino acids of human IL-6. These antibodies reacted with recombinant IL-6 by ELISA and stained the cytoplasm of the IL-6 secreting bladder tumour cell line T24. Staining was abolished by prior incubation of the antibody with the IL-6 peptide. F(ab')2 fragments made by pepsin digestion of the IgG were immunopurified, labelled with biotin and retained activity in the biochemical and histological assays. Sections of synovial membrane from patients with rheumatoid arthritis (RA) were stained with these antibodies, using an immunoperoxidase technique, and cells containing IL-6 were demonstrated in the thickened synovial lining layer and also in a perivascular distribution in the deeper synovium. In osteoarthritis there were fewer cells in the lining layer and hence localisation appeared similar in both the interstitial area and lining layer. Double-staining techniques with mouse monoclonal antibodies against cell subset markers in five RA synovial membranes showed that up to 13% of T-cells and 19% of antibody-producing cells stained for IL-6. However, up to 70% of the macrophages contained IL-6 and these were found in close proximity to Ig-producing plasma cells. This study showed that macrophages were the major cells of the immune system in which IL-6 could be localised in RA, and suggests a role for locally produced IL-6 in the stimulation of rheumatoid factor production.

Key words: Interleukin-6 - Rheumatoid arthritis - Synovial membrane - Immunohistology - Autoimmune disease

Introduction

Interleukin-6 (IL-6) is the currently accepted name for the cytokine originally termed interferon β2, B-cell Stimulation Factor-2, hepatocyte stimulation factor or hybri-
in the inflamed joint, and to investigate whether this molecule could be playing an important role in the autoimmune process in RA.

Materials and methods

Antibody production. Rabbit polyclonal antibodies were raised as described [3] using a synthetic peptide of IL-6 (12 Amino acids of identical sequence to the NH\textsubscript{2} terminal portion) linked to thyroglobulin (donated by Dr. J. Rothbard, Imperial Cancer Research Fund (ICRF)) in a standard immunisation protocol. Subsequent boosts were performed using a yeast supernatant containing human IL-6 (Donated by Immunix, Seattle). Serum was separated and stored at –20°C prior to use. Anti-sera from each rabbit were screened by ELISA using recombinant IL-6 (donated by Drs. Kishimoto and Hirano) with an anti-rabbit IgG linked to alkaline phosphatase (Sigma, Poole, Dorset, UK) followed by Sigma 104 substrate.

Serum containing anti-IL-6 antibodies was digested using pepsin (Sigma) to produce F(ab')\textsubscript{2} fragments and undigested IgG removed using Staphylococcal protein A sepharose (Pharmacia, Milton Keynes, UK) and stored at -70°C until used. They were fixed in Aceton-Methanol 1:1 and non-specific adherence was inhibited by (20 gg/ml) was incubated with the cells for 90 mins and detected using a streptavidin linked to fluorescein conjugate (Amersham) a dilution of 1:50 for 1 h. These were either visualised using a Laborlux 12 microscope (Leitz, Luton, Beds., UK). Macrophages were detected with a cocktail of UCHM1, clonal antibody 63D3 which recognises a 200 K protein on macrophages [18], which as yet has no CD number (both donated by Dr. P. Beverley). These were detected using anti-mouse immunoglobulin linked to Texas red at a dilution of 1:50 (Sigma). To detect cells in the B-cell lineage (including plasma cells), a goat F(ab')\textsubscript{2} fragment against human F(ab')\textsubscript{2} directly conjugated to fluorescein (Kallestad, Austin, Tex., USA) was used (dilution of 1:100) with a streptavidin linked to Texas red to detect the anti-IL-6 F(ab')\textsubscript{2} fragments. To detect rheumatoid factor producing plasma cells, mouse monoclonal antibodies against human F(ab')\textsubscript{2} fragments derived from rheumatoid factors were used (donated by Dr. R. Jeffries) followed by anti-mouse Ig linked to fluorescein as described above.

Results

Antibodies shown to react with IL-6 by ELISA were screened on the T24 cell line. This demonstrated a cytoplasmic staining pattern (Fig. 1 a) which was inhibited by prior incubation of the antibody with IL-6 peptide prior to screening on the T24 cells (Fig. 1 b), confirming antibody specificity by histological means. Biotin labelled F(ab')\textsubscript{2} anti-IL-6 antibody fragments produced an identical staining pattern (data not shown).

Staining of the seven synovial membranes from patients with RA shows cells containing IL-6 in two major sites. Firstly they can be seen in the interstitial tissue close to blood vessels in the synovium. In addition, some blood vessel endothelial cells also stained with these antibodies (Fig. 2 a). Other cells were detected close to and in the lining layer by staining with these antibodies (Fig. 2 b), and one patient showed a multinucleate cell staining for IL-6 (not shown). Seven patients with RA were examined and 30.9% of the cells in the lining layer of the RA patients were found to contain IL-6 (Table 1), whereas 2.3% of the cells in the deeper interstitial tissue were positive for IL-6. No staining was observed with an irrelevant rabbit F(ab')\textsubscript{2} fragment raised against the rat allotype OX12 (Fig. 2 c).