LYMPHOCYTE-SYNOVIAL MICROVASCULAR ENDOTHELIAL CELL INTERACTIONS IN EXPERIMENTAL POLYARTHRITIS

: A microassay for screening monoclonal antibodies that block adhesion

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ABSTRACT

Although the causes of rheumatoid arthritis (RA) are still not well defined, an improved understanding of the pathogenesis of this disease has the potential to allow the development of new and innovative therapies. An understanding of each facet of the adhesion cascade leading to T cell migration into synovium should help to identify molecules that are involved directly with propagation of synovial inflammation. By targeting these molecules, treatments might be developed that are more efficacious, specific and less toxic systemically than current therapies. Furthermore, if adhesion molecules can be identified that have expression limited to synovium, novel therapeutic approaches aimed at blocking the function of adhesion molecules offer advantages in the management of arthritis. Thus, offering an immunotherapy for arthritis that is limited in its systemic effects on the immune system

With this in mind, an experimental system was developed to allow direct investigation of the adhesion of activated lymphocytes to microvascular endothelial cells (EC) from the inflamed synovium of rats with adjuvant arthritis (AA). The experimental system that was developed is novel, in that the EC were prepared from the microvasculature as primary cultures. Previous studies, utilising various models, have highlighted a role for a number of adhesion molecules in the recruitment of inflammatory cells during the pathogenesis of immunologically mediated polyarthritis. Earlier *in vitro* studies used either EC derived from large vessels, such as the rat aorta or the human umbilical cord, or from the microvasculature of non-articular tissues such as the normal rat heart. EC populations from normal tissues have required *in vitro* stimulation with pro-inflammatory mediators prior to use in an adhesion assay, in order to upregulate expression of adhesion molecules. In contrast, the EC used in the experimental system described herein are derived from synovium and they have been activated *in vivo* during the pathogenesis of polyarthritis. It was possible, therefore, to investigate the adhesive interactions between freshly isolated populations of synovial microvascular ECs that were activated *in vivo* and activated T cells. Two different sources of activated T cells were used. They were T cells from lymph nodes activated *in vitro* with Con A and *in vivo* activated CD4+ T cells obtained from the thoracic

duct (TD) lymph of donor rats during the late prodromal period of adjuvant induced arthritis. Cells in thoracic duct lymph during this phase of AA are known to enter inflamed synovium and they can transfer the disease adoptively.

This project investigated lymphocyte-EC adhesive interactions in a novel assay system. The molecular basis for lymphocyte-EC adhesion was investigated using an existing panel of monoclonal antibodies against rat adhesion molecules. The study has demonstrated a role of adhesion molecules CD54, CD11a/CD18, CD49d, CD62L and CD62P in the adhesion lymphocytes from both sources, to synovial microvascular EC. In addition, hybridomas were generated from the spleen of mice immunised with synovial cells produced from rats with established AA. Two hybridoma supernatants have been identified that contain antibodies that significantly inhibit lymphocyte adhesion to synovial microvascular EC monolayers. The results indicate that the microassay of lymphocyte–EC adhesion that has been developed in this work has wider application in characterising new monoclonal antibodies against adhesion molecules and could also be used to explore interactions between immune cells and EC from other sites of inflammation.

Development of a technique for preparation and purification of EC from synovium has allowed studies of other cells in synovial tissues. Lymphocyte subsets found in the synovim have been defined and expression of activation markers and adhesion molecules expressed by T cells have been examined by flow cytometry. Most of the T cells recovered from this tissue belong to the CD4+ subset and they express activation markers and elevated levels of the adhesion molecules: CD44, CD11a, CD54, CD49d, MHC class II, CD71 and CD25 accompanied by low levels of CD45RC and CD62L. This phenotype is consistent with that of effector T cells. The method for producing a mixed cell suspension from rat synovial tissue has shown great utility in studies on the pathogenesis of polyarthritis.

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