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Localization of hyaluronan and the hyaluronan receptor ICAM-1 in rheumatoid synovia—a histochemical study

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The major site for elimination of HA from the bloodstream is via receptor mediated endocytosis by liver endothelial cells (LEC) (Fraser and Laurent 1989, Laurent and Fraser 1992). The HA receptor (HAR) on LEC has been characterized and isolated from rat LEC membranes (Forsberg and Gustafson 1991). A monospecific polyclonal antibody against the 90–100 kD receptor, which could inhibit binding of HA to LEC and LEC membranes, (termed anti-HARLEC) was raised (Forsberg and Gustafson 1991). In immunohistochemical studies the antibody stains mainly endothelium such as the sinusoids in the liver, spleen and lymph nodes, some capillaries in kidney and corneal endothelial cells (Gustafson et al. 1994, Forsberg et al. 1994). Tryptic peptides of the receptor were sequenced and found to be identical to intercellular adhesion molecule-1 (ICAM-1) (McCourt et al. 1994). ICAM-1 is normally expressed at low levels, but has been found on normal liver endothelium at the sinusoids and on the endothelium of lymph nodes, spleen and some capillaries of the kidney (Dustin et al. 1986), as well as on corneal endothelial cells (Foets et al. 1992). The localization corresponds well with anti-HARLEC staining (Gustafson et al. 1994, Forsberg et al. 1994) and to tissues where HA binding and uptake have been found (Fraser and Laurent 1989, Laurent and Fraser 1992, Forsberg et al. 1994). ICAM-1 is upregulated in inflammation and neoplasia and is a key molecule for leukocyte adherence and transendothelial migration (Dustin et al. 1986, Tamatani and Miyasaka 1990, Springer 1994). We have recently also shown that HARLEC/ICAM-1, expressed on tumor endothelium in mouse mastocytomas, can bind intravenously administered radiolabeled HA (Gustafson et al. 1995).

The aim of this study was to investigate the relationship between HA and ICAM-1 in human rheumatoid synovia.

Materials and methods

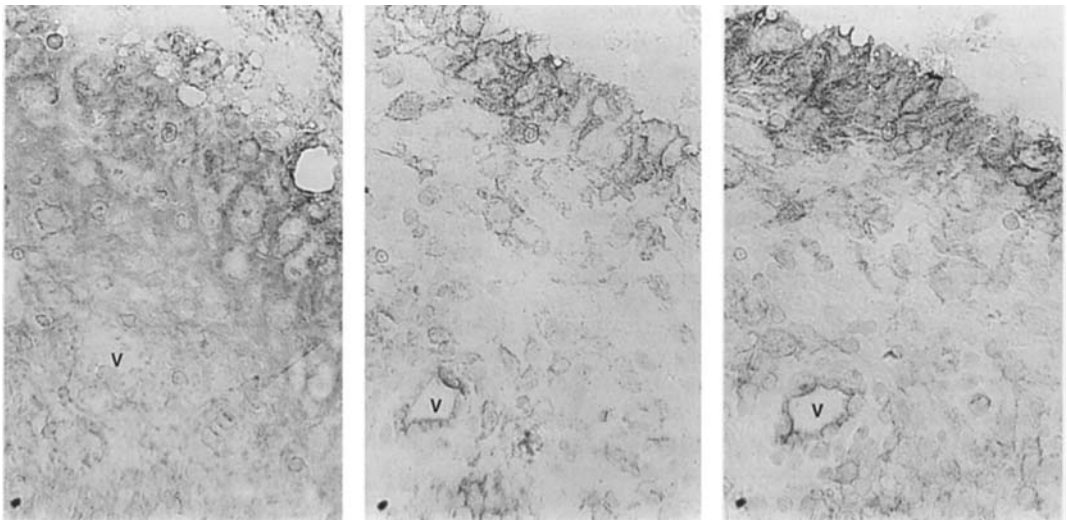
Monoclonal antibodies: The monoclonal antibody against human ICAM-1, clone 15.2, was from Southern Biotechnology, Birmingham, U.S.A., and the monoclonal antibody towards human CD68 was from DAKO, Glostrup, Denmark.

Tissues: Rheumatic synovial membrane from patients suffering from classical seropositive rheumatoid arthritis were collected at therapeutic orthopedic synovectomies.

Immunostaining: Frozen sections of 6 µm were prepared and mounted on glass slides that were coated with gelatin-kromalun. The frozen sections were fixed in cold methanol for 10 minutes, dried for 10 minutes before being washed with phosphate buffered saline (PBS). Endogenous peroxidase was blocked in 0.3% H_2O_2 in methanol, and the sections were once more washed in PBS. To block endogenous biotin and biotin-binding activity, an avidin/biotin blocking kit from Vector laboratories was used. The sections were then incubated for 30 min in PBS containing 4 % horse serum (Vectastains® Elite ABC). The monoclonal antibody was diluted 1: 300 in 4% horse serum in PBS, and incubated for one hour. After washing in PBS, the sections were incubated with the secondary HRP-conjugated horse anti-mouse antibody (Vectastaine® Elite ABC) diluted 1:200 in PBS for 30 min.

The staining for HA was performed as described previously (Gustafson et al. 1995), using biotinylated hyaluronan binding proteins from cartilage (b-HABP). After incubation with the second antibody, or b-HABP, the sections were washed and incubated

Figure 1. Frozen sections of rheumatoid synovia stained for HA and ICAM-1. See Materials and Methods for details. Original magnification 40x. V denotes vessel.



Staining for HA

Staining for ICAM-1.

Staining for ICAM-1 after hyaluronidase treatment.

with the ABC-Elite-complex (Vectastain® Elite ABC). To develop the color, peroxidase substrate kits (Vector laboratories) containing 3,3 diaminobenzidine or 3-amino-9-ethylcarbazole were used as described previously (Gustafson et al. 1994, Gustafson et al. 1995)

Hyaluronidase treatment of sections: After methanol fixation and washing in PBS the sections were incubated with 5 U/ml of streptomyces hyaluronidase (Amano pharmaceutical Co., Ltd Japan.), 1.8 µg/ml pepstatin (Sigma), 1.8 mM EDTA (Merck), 1.8 µg/ml soybean trypsin inhibitor (Sigma), 2.0 mM iodo acetic acid (Sigma), 0.18 mM E-amino-caproic acid (Sigma) and 9.0 mM benzamide (Sigma) for two hours at 37 °C. The regular staining protocol was then followed.

Results

When synovial tissue from humans suffering from rheumatoid arthritis was analyzed by histochemical techniques, it was found that the HA and ICAM-1 was mainly localized to the synovial lining cells (Figure 1a and b). These cells also stained positive for CD68 (not shown), indicating that these HA and ICAM-1 positive cells are macrophages. ICAM-1 staining was also found in vessels (Figure 1b). After hyaluronidase treatment the staining for ICAM-1 was dramatically increased on the synovial lining cells, but was not significantly increased in vessels (Figure 1b and c).

Discussion

The finding that monoclonal antibodies to ICAM-1 predominantly stain the macrophage related synovial lining cells (SLC) of rheumatoid synovia, colocalizes with the staining for HA, and is dramatically increased after hyaluronidase treatment, indicates that HA is bound to ICAM-1 on the SLC. The relatively weak immunostaining of the SLC observed prior to hyaluronidase treatment, indicates that the majority of the HA binding sites on ICAM-1 are occupied by endogenous HA. Synovial fluid has been reported to contain high levels of HA (Engström-Laurent 1989), and this fact, together with the low level of unoccupied ICAM-1 on the lining cells, makes it likely that intraarticular injections of HA will have only limited effect in cartilage disease, as indeed has been reported. (Dahlberg et al. 1994).

The lack of a significant increase in ICAM-1 staining of vessel endothelium after hyaluronidase treatment suggests that the HA binding sites on ICAM-1 at this location are predominantly unoccupied, probably in part due to the low levels of circulating HA, and could possibly be targeted by systemically delivered exogenous HA, as has been shown for tumor endothelium (Gustafson et al. 1995). Targeting and binding of HA to endothelial cells expressing ICAM-1 could explain the inhibition of acute inflammation as well as chronic inflammation in adjuvant arthritis seen after systemic administration of HA in animal models (Ialenti et al. 1994). The mechanism could be

based on a competition between HA and leukocytes for ICAM-1, resulting in suppression of leukocyte infiltration into the inflamed tissues. It is interesting to note that therapy with monoclonal antibodies to ICAM-1 has similar effects to systemic HA therapy in animals (Iigo et al. 1991).

Our previous findings that HA binds to endothelial cells via ICAM-1 and the present study, showing ICAM-1 on vessel endothelium of rheumatic synovia that is apparently free from significant amounts of endogenous HA, points to the possibility of using HA as an immunomodulator in inflammatory conditions by competition with leukocytes for ICAM-1. HA could possibly also be used to carry drugs to pathological sites expressing this type of HA-receptor.

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