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Intercellular adhesion molecule 1 (ICAM-1) induction on hepatocytes is an early marker of acute liver allograft rejection

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Abstract. Intercellular adhesion molecule 1 (ICAM-1) induction on hepatocytes was investigated in relation to immune activation of acute liver allograft rejection. Twelve liver recipients undergoing an episode of acute rejection were monitored by frequent fine needle aspiration biopsy (FNAB) study. All episodes were reversible, and the lymphocyte and lymphoid blast predominated with a high peak of inflammation (6.9 ± 4.0 corrected increment units). The rejections were treated with a high dose of steroids, and the inflammation subsided within 1 week. ICAM-1 was demonstrated from FNAB preparations by a monoclonal antibody and immunoperoxidase staining. ICAM-1 was not detected on the hepatocytes immediately after transplantation but was always seen during rejection. ICAM-1 appeared 1–5 days before the onset of inflammation in the FNAB. The intensity of ICAM-1 expression increased towards the peak of inflammation and subsided thereafter. ICAM-1 induction on hepatocytes appears to be linked with a very early phase of immune activation and can be considered an early marker for acute liver allograft rejection in the FNAB.

Key words: Liver allograft rejection – ICAM-1 induction – Hepatocytes

Intercellular adhesion molecule 1 (ICAM-1), a ligand for lymphocyte function antigen (LFA-1), is a cell surface glycoprotein with important functions in leucocyte adhesion and the cell-cell interactions of the inflammatory processes [1]. The expression of ICAM-1 is upregulated by several cytokines, such as IL-1, TNF-α and INF-γ, and it is produced at the early phase of lymphoid activation [2]. Expression of ICAM-1 on hepatocytes has also been demonstrated on biopsy histology during liver allograft rejection [3, 4]. Although the role of adhesion molecules in liver rejection is not yet clear, they are upregulated by the cytokines produced during the cascade of immune activation of acute rejection.

The hallmark of acute liver allograft rejection monitored by the fine needle aspiration biopsy (FNAB) method is the appearance of a lymphocyte and lymphoid blast-dominated inflammatory infiltrate in the graft [5]. The cellular findings in the FNAB correlate with clinical signs and biochemical markers of acute liver rejection, but the cellular hallmarks of rejection are usually seen 1–3 days before the clinical diagnosis of rejection is established [5].

In this study the ICAM-1 induction on hepatocytes was investigated in relation to the immune activation of acute liver allograft rejection monitored by frequent FNABs. The induction of ICAM-1 was correlated with the appearance of lymphoid activation in the graft, and the downregulation of ICAM-1 expression was correlated with the disappearance of inflammation during anti-rejection treatment.

Patients and methods

Patients. Twelve liver transplant recipients treated with combinations of azathioprine (1–2.5 mg/kg daily), cyclosporine (3–10 mg/kg daily) and methylprednisolone (0.5–2 mg/kg daily) for basic immunosuppression (triple therapy CyA, Aza, MP) underwent an inflammatory episode of acute rejection monitored by fine needle aspiration biopsy (FNAB). The recipients received high-dose MP (3 mg/kg daily) as the anti-rejection therapy for 5 days. All rejections were reversible with steroids. No infections occurred during the rejection episodes.

FNAB and blood specimens. The liver allografts were monitored with FNAB from the day of transplantation at 1 to 3-day intervals. The method for performing and processing FNABs of liver [6] and corresponding blood specimens is similar to that described for renal allografts [7]. The inflammation associated with rejection was quantified from May-Grunwald-Giemsa (MGG) stained cytocentrifuge preparations by the increment method and expressed in corrected increment units (CIU) [7].

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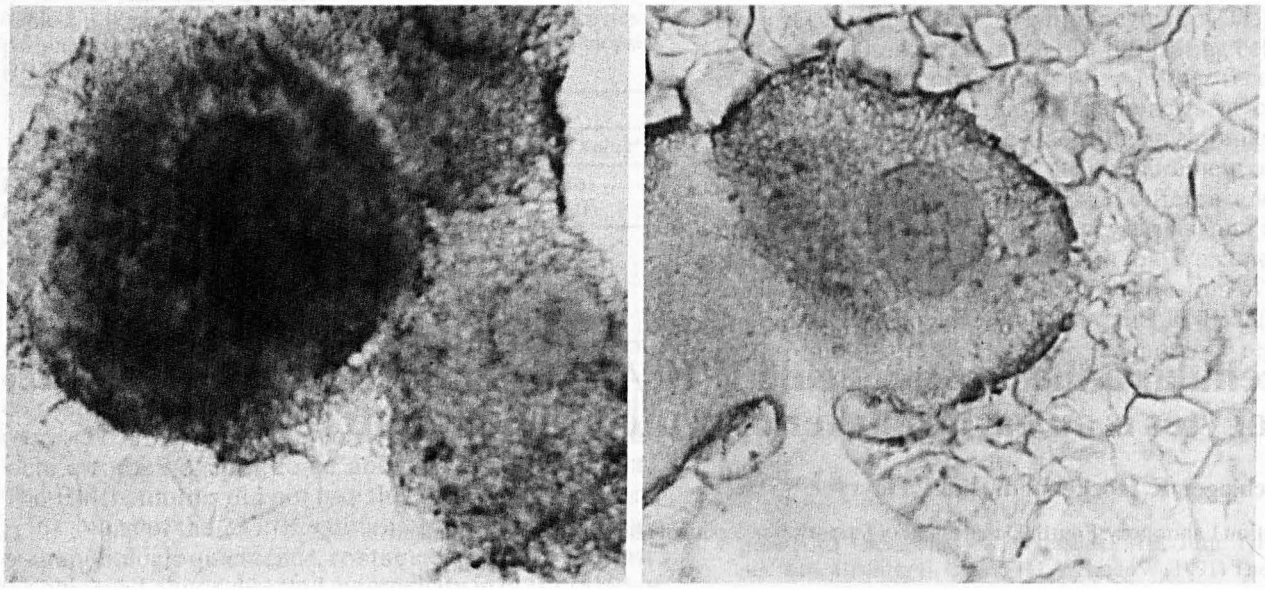

Fig. 1 a, b. An intense expression of intercellular adhesion molecule 1 (ICAM-1) on hepatocytes (score 3) at the peak of lymphoid activation (**a**), and a negative for staining ICAM-1 of hepatocytes after the inflammatory episode of rejection (**b**) demonstrated by monoclonal antibody and immunoperoxidase techniques

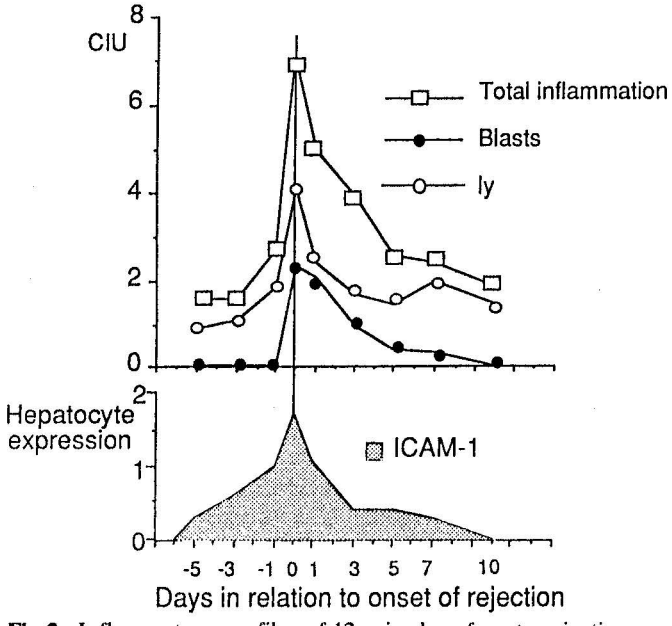

Fig. 2. Inflammatory profiles of 12 episodes of acute rejection expressed in corrected increment units (CIU). The major inflammatory cell components of total inflammation are lymphocytes *(ly)* and lymphoid blast cells. The induction and the intensity of ICAM-1 expression (scored from 1 to 3) is demonstrated in relation to onset of immune activation of rejection seen in the biopsy study

Demonstration of ICAM-1. For further analysis of ICAM-1 expression, a three-layer indirect immunoperoxidase technique and a monoclonal antibody to ICAM-1 were used. A monoclonal antibody against CD54 (ICAM-1) (Immunotech, Marseille, France) was employed. The cytocentrifuge preparations of liver FNABs were first incubated with the monoclonal mouse antibody, then with the peroxidase-conjugated rabbit antimouse antibody (Dako, Copenhagen, Denmark) and thereafter treated with a peroxidase-conjugated goat antirabbit antibody (Tago, Burlingame, Calif.). The reaction was revealed by AEC (3-amino-9-ethyl carbatzole) solution containing hydrogen peroxide. Mayer's hemalum was used for counterstaining. The intensity of positive staining was scored from 1 to 3.

Results

The rejection episodes appeared during the first postoperative month (6–27 days). All episodes demonstrated a high peak of inflammation (1.6 ± 0.8 CIU before, 6.9 ± 4.0 CIU at the peak and 1.9 ± 0.9 CIU after the episode), and subsided within 1 week. The inflammatory infiltrate consisted mainly of lymphocytes (0.9 ± 0.6 CIU before, 4.1 ± 1.8 CIU at the peak and 1.5 ± 0.9 CIU after the episode) and lymphoid blast cells (2.3 ± 2.5 CIU at the peak), with a minor involvement of mononuclear phagocytes. All rejection episodes were reversible, and the inflammation subsided with a high dose of MP in 5–7 days.

ICAM-1 was not detected in the FNABs obtained immediately after transplantation but was always seen during rejection. The expression of ICAM-1 on hepatocytes was induced 1–5 days before the onset of inflammation. The intensity of ICAM-1 expression (scored from 1 to 3) increased towards the peak of inflammation (0.3 ± 0.5 on day 5, 0.6 ± 0.7 on day 3, 1.0 ± 0.6 on day 1 before the onset and 1.7 ± 0.8 at the peak of inflammation) and subsided slowly thereafter. Some expression of ICAM-1 (0.3 ± 0.6) was still recorded on day 7, but absolutely negative staining for ICAM-1 was always seen 10 days after the onset of rejection and the administration of anti-rejection therapy (Fig. 1). The cytological findings of lymphoid activation in acute liver allograft rejection thus closely correlated with the intensity of ICAM-1 on hepatocytes. However, ICAM-1 induction on hepatocytes preceded the other markers of rejection in the FNAB (Fig. 2).

Discussion

The expression of ICAM-1 on hepatocytes has been demonstrated in biopsy histology during liver rejection [3, 4]. In our study, the frequent FNAB monitoring made it possible to investigate the induction of ICAM-1 expression in relation to immune activation of liver allograft rejection. ICAM-1 induction was recorded at a very early phase of immune activation in the graft. The up-regulation of ICAM-1 even preceded the lymphoid activation.

ICAM-1 and its complementary adhesion molecule LFA-1, expressed on lymphocytes and on other leukocytes, are important in the cell-cell interactions and in T-cell activation at the early phase of inflammatory processes. Also, the antigen presentation of the cells is dependent on ICAM-1, in addition to major histocompatibility complex (MHC), and co-expression of those molecules is needed for T-cell activation [8].

The role of adhesion molecules in the anti-allograft response is thus obvious, as well as the induction of those molecules during the immune activation of rejection. On the other hand, ICAM-1 expression has also been demonstrated in biopsy histology of liver from recipients with bacterial and viral infections [4] and may thus be considered as an unspecific marker of immune processes. Though unspecific, ICAM-1 induction on hepatocytes is an early marker for acute liver allograft rejection in the FNAB.

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