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Early intragraft inflammatory events of liver allografts leading to chronic rejection

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Abstract In this retrospective study, we have investigated the early intragraft inflammatory events of 12 liver allografts leading to chronic rejection. The cytological findings and clinical follow-up were analyzed in detail. Nine patients underwent at least one typical lymphoid activation of acute rejection, and three of them were treated more than once. Diagnosis of rejection was based on biopsy histology, cytology and liver dysfunction. In addition to the acute rejections, cytological analysis demonstrated in 11 of 12 grafts an unidentified lymphoid episode that differed from that of rejection. These lymphoid responses were associated with viral infections; cytomegalovirus (CMV) infection in 10 of 12 patients, hepatitis C virus (HCV) infection in 2 of 12 patients, 1 combined with CMV, and hepatitis B virus (HBV) infection in 1 patient. Graft dysfunction was still seen at the end of the follow-up. Thus, intragraft inflammation caused either by acute rejection or by viral infections may be involved in the induction of chronic rejection.

Key words Liver transplantation, chronic rejection · Rejection, liver transplantation · Viral infections, liver transplantation

Introduction

Chronic rejection of the liver transplant is clinically characterized as a slow-progressing and relatively asymptomatic process with increasing graft dysfunction that occurs in about 10 % of liver allograft recipients [6, 17]. Histologically chronic liver allograft rejection usually manifests itself as vanishing bile duct syndrome (VBDS), which includes the disappearance of bile ducts and vascular changes, such as obliterative arteritis [5, 14, 19]. Only a relatively mild lymphocytic infiltration may be seen in the portal areas, or the inflammation may even be absent. Loss of bile ducts may also occur without the characteristic histological findings of occlusive arterial lesions, and arterial changes of chronic rejection may be present without VBDS [4].

In general, a common manifestation of chronic rejection in various organ allografts is persistent perivascular and interstitial inflammation with a low activation level of lymphocytes [9]. This suggests that an allogenic injury to transplant arteries and possibly to other structures may be further aggravated by other risk factors, the primary pathogenetic mechanism of chronic rejection. However, the inflammatory infiltration and immunological mechanisms seem to be necessary for the process to lead to the characteristic findings of chronic rejection in the organ allografts [9].

The etiology of chronic rejection is multifactorial [9]. Among the risk factors are histocompatibility, the frequency of acute rejection episodes, a long ischemia time, and infections [9]. An association between viral infections, especially cytomegalovirus (CMV) infection, and chronic rejection has been suggested. CMV is a major infectious agent complicating the postoperative course of liver transplant patients, and an association between VBDS and CMV infection has been reported [16]. The persistent appearance of CMV DNA in hepatocytes has been linked with chronic liver rejection [1]. Also,

other viral infections, such as hepatitis B (HBV) and hepatitis C (HCV), may mimic the histological changes of chronic rejection, although it is not known whether they are involved in the pathogenesis of VBDS [22].

In this retrospective study, we have investigated in detail the early postoperative inflammatory events of liver allografts that lead to irreversible chronic rejection. A detailed analysis of inflammatory infiltration of the graft was based on fine-needle aspiration biopsy (FNAB) monitoring and transplant aspiration cytology (TAC) material [11, 18]. The cellular specimens were blindly analyzed without any knowledge of the clinical course of the patients, and the cytological findings were retrospectively correlated with the clinical events. The aim of the study was to investigate the intragraft events and possible induction of the slow pathological processes leading to chronic rejection.

Patients and methods

Patients

Until the end of September 1993, 868 liver transplantations were performed on 731 patients at the Clinic of Abdominal and Transplantation Surgery, Medical School Hannover, Germany. The overall frequency of irreversible chronic rejection was 4.8% (29/603) in adult patients. Since 1988 liver allografts of adult recipients have been monitored with fine-needle aspiration biopsy (FNAB) and transplant aspiration cytology (TAC) from the moment of transplantation until 2–3 months postoperatively [11, 18]. Of the patients who have been cytologically monitored since 1988, there have been 12 cases leading to histologically confirmed chronic rejection. The FNAB material demonstrating the intragraft inflammatory events and the clinical follow-up of these 12 patients were analyzed in detail retrospectively.

Eight patients had undergone liver transplantation for posthepatitis cirrhosis: five of them were diagnosed with posthepatitis B, two with non-A non-B hepatitis, and one with hepatitis C. Two patients had primary biliary cirrhosis, one Budd-Chiari syndrome, and one sclerosing cholangitis. Chronic rejection with vanishing bile ducts appeared 2–24 months (mean 9 months) after transplantation. There was no significant association between HLA matches, either MHC class I or class II, and the appearance of chronic rejection. The cold ischemia time ranged from 5 h 38 min to 20 h 18 min (mean 13 h 36 min).

Basic immunosuppression consisted of triple or quadriple therapy, combining steroids, cyclosporin A, azathioprine and antithymocyte globulin (ATG). Rejection episodes were treated with high doses of methylprednisolone (MP), and in the case of steroid resistant rejection, OKT3 was administered. The diagnosis of acute rejection was based on the clinical picture, biopsy histology, biochemical markers of rejection, and cytological monitoring of the graft. The diagnosis of chronic rejection was based on biopsy histology.

Transplant aspiration cytology

The FNAB specimens were blindly reanalyzed in detail retrospectively without any knowledge of the clinical course of the patients. FNAB samples were routinely obtained at 1- to 5-day intervals during the first 2–3 months after transplantation. The method used to perform and to process the liver FNABs and the corresponding blood specimens was the same as that used for kidney allografts [8] and has been described previously [11, 12, 18]. The cytocentrifuge preparations of the specimens were stained with Romanowsky-Giemsa stain [18]. The inflammation was quantified from the cellular smears using the increment method and expressed in corrected increment units (CIU) [8, 11]. To evaluate numerically the intensity of inflammation from the smears by the increment method, differential counts were done on both the FNAB and blood specimens, and correcting factors were used according to the diagnostic value of each inflammatory cell type [8, 11]. Thus, lymphoid blasts had the highest correction factor, 1.0, activated lymphocytes 0.5, large granular lymphocytes (LGL) 0.2, small lymphocytes 0.1, polymorphonuclear cells 0.1, monocytes 0.2, and macrophages 1.0. The intensity of inflammation in the graft was expressed in CIU, as a total increment, which is the sum of CIU values of aspiration differential after subtracting the blood background. An inflammation equal to or greater than 3.0 CIU, together with lymphoid blast cells in the FNAB differential, was considered the beginning of immune activation in the graft [11, 12]. Altogether, 164 FNABs and corresponding blood specimens, all obtained from the 12 patients during the first 3 postoperative months, were analyzed in detail and included in this study.

Results

FNAB monitoring demonstrated inflammatory episodes and lymphocytosis in every graft during the first 3 post-transplant months. Two types of inflammatory episodes were recorded. Acute rejections with a typical lymphoid activation and blast response appeared in nine grafts. In addition, another type of lymphoid response with significant lymphocyte infiltration, with or without slight activation, was recorded in 11 of the 12 allografts. This unidentified lymphoid infiltration was, in every case, due to an episode of viral infection. All 12 patients had viral infections during the 2–3 postoperative months. CMV infection was diagnosed in ten cases, four of which were treated with ganciclovir. Two patients developed HCV reactivation, in one case combined with CMV infection, and one patient had HBV infection. Six patients also had other infectious complications, including systemic bacterial infections, and two had candidiasis. Those infections did not have any effect on the cellular picture of transplant aspiration cytology.

Acute rejections

The diagnosis of acute rejection was based on biopsy histology and cytology and clinical evidence of graft dysfunction. Nine out of 12 patients had at least one episode of acute rejection during the first 3 postoperative months; three of them had more than one episode. Three rejections had to be treated with OKT3, and in one case FK 506 was administered after the steroid ther-

apy. All acute rejections were reversible. The cellular findings of allograft rejection, monitored with FNAB, were typical, with a high peak of inflammation (5.4 ± 1.7 CIU), dominated by activated lymphocytes and lymphoid blasts in the graft. The inflammation subsided with successful antirejection therapy.

Lymphoid responses other than rejection

Retrospective analysis of clinical data showed that all 12 patients had viral infections during the first 2–3 months after transplantation. The unidentified lymphoid response in 11 of 12 grafts was found to be associated with viral infection. In ten patients, an episode of CMV infection was diagnosed by IgM seroconversion at the time of lymphoid response. In one case of CMV infection, no FNAB monitoring was done during the diagnosis. In four cases the clinical symptoms were considered severe and the patients were treated with ganciclovir. HCV infection was associated with the lymphoid response in two cases, probably reactivations. One of them also had a CMV reactivation at the same time, and HBV infection was the underlying cause of the lymphocytosis in one patient.

Lymphoid responses associated with viral infections differed strikingly from those of rejection. With viral infections, the cellular picture was dominated by small lymphocytes in the graft, with minor lymphoid activation and almost no blast response. However, total inflammation even exceeded the level of rejection (5.5 ± 1.1 CIU). A peak of lymphoid infiltration of the graft occurred together with seroconversion, indicating viral infection. The lymphoid response caused by viral infections subsided within a few days or weeks; this was also true in the cases of untreated CMV infections and HCV reactivations or HBV infections.

In 7 of the 12 patients, a rejection episode and antirejection therapy preceded viral infection (CMV in all 7 patients). Viral infection appeared without preceding antirejection treatment in 5 of the 12; in the case of HBV infection, it occurred at the same time. Rejection episodes followed viral infections in two patients, both of whom had CMV infection. Figures 1 and 2 present a detailed, cellular analysis of two patients with viral infection, one with HCV reactivation and another with CMV infection preceding acute rejection.

Graft function

Graft dysfunction was recorded during both acute rejections and at the peak of inflammation due to viral infections. Elevated values of serum transaminases (ASAT 326 ± 451 U/l, ALAT 387 ± 259 U/l), alkaline phosphatase (591 ± 542 U/l), bilirubin (241 ± 136 μmol/l)

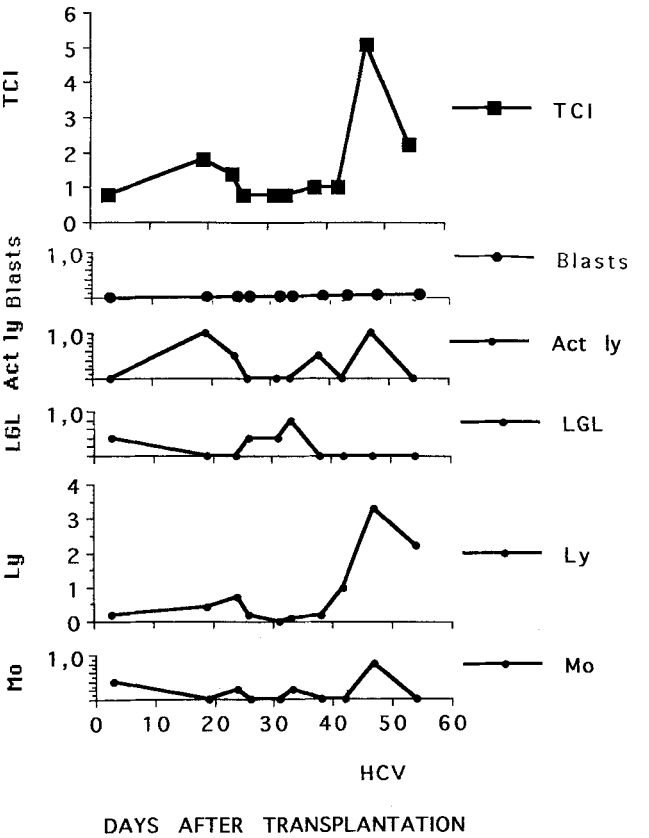

Fig. 1 The cellular profiles of intragraft inflammatory infiltration, expressed in corrected increment units (*CIU*), of a patient with HCV infection. The peak of inflammatory infiltration of total inflammation (*TCI*) consisted mainly of small lymphocytes (*Ly*) with a few activated lymphocytes (*Act ly*) and with minor involvement of monocytes (*Mo*) and large granular lymphocytes (*LGL*). No lymphoid blasts (*Blasts*) are seen

and γ-GT (325 ± 268 U/l) were seen during the rejection episodes. The levels of serum transaminases (ASAT 97 ± 73 U/l, ALAT 200 ± 175 U/l) and bilirubin (129 ± 124 μmol/l) were lower with viral infections, but alkaline phosphatase (1110 ± 524 U/l) and γ-GT (401 ± 316 U/l) were higher. The differences were not significant. Elevated serum values of biochemical parameters (ASAT 154 ± 298 U/l, ALAT 172 ± 171 U/l, alkaline phosphatase 1088 ± 553 U/l, bilirubin 195 ± 187 μmol/l, and γ-GT 419 ± 316) were still seen in all patients at the end of the 2- to 3-month post-transplant monitoring period. Impaired graft function led to a diagnosis of chronic rejection within 1–7 months in 8 of the 12 patients; the other 4 patients developed chronic rejection within 1–2 years after transplantation.

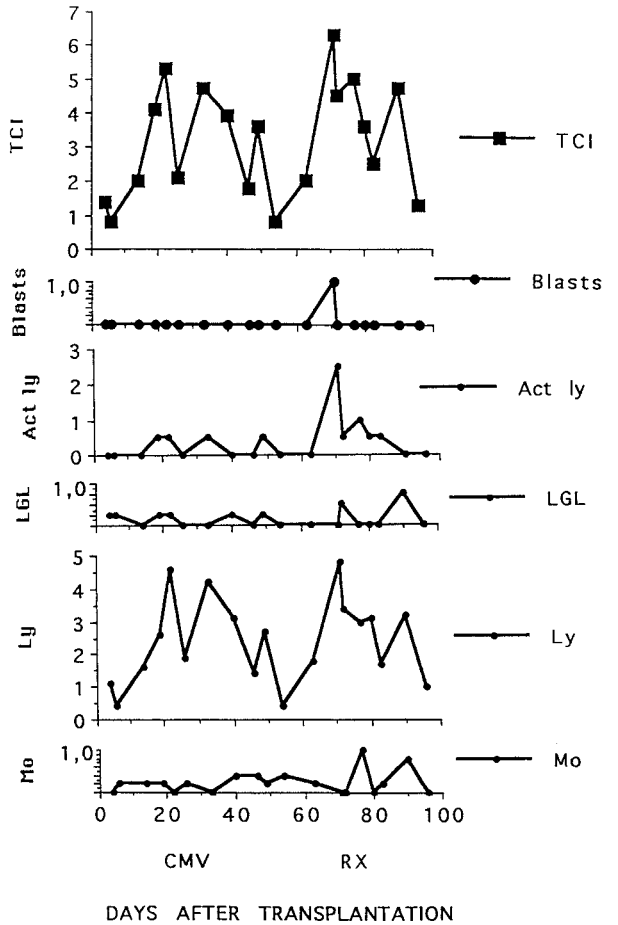

Fig. 2 A significant inflammatory episode of CMV infection preceding a prolonged, severe acute rejection. The inflammatory infiltration during CMV infection consisted mainly of small lymphocytes (*Ly*), with a few activated cells (*Act ly*), large granular lymphocytes (*LGL*), and monocytes (*Mo*). The inflammation associated with acute rejection is typical, with lymphoid blast response (*Blasts*), a large number of activated lymphocytes, and a minor increase in LGL cells and monocytes

Discussion

The detailed cytological follow-up of 12 liver transplant recipients who eventually developed chronic rejection demonstrated lymphocyte-dominated inflammatory episodes in every graft during the early postoperative course. In addition to acute rejection episodes, which occurred in an exceptional number of recipients (9/12), every patient (12/12) had a viral infection during the first 3 post-transplant months. The viral infections were associated with an intragraft lymphoid response that differed from the typical cellular picture of acute rejection [12, 13, 18]. Cellular infiltration was dominated by small lymphocytes; almost no lymphoid activation or blast response was recorded during the viral infections.

The most common viral infection was CMV, occurring in 10 of the 12 grafts; however, HCV and HBV were also involved. As has previously been suggested, CMV may trigger the rejection cascade, possibly by increasing the lymphoid immune response against the virus. Lymphoid activation, especially the activation of Th1 cells [3], leads to the production of various cytokines, such as interleukins and interferon-γ, and to the upregulation of MHC antigens [1, 13, 21]. This immunological triggering may be a mild and slow process that leads to chronic allograft rejection [9]. It is also possible that other viruses that affect the liver graft and increase an intragraft lymphoid response, such as hepatitis viruses, may trigger the immunological cascade of chronic rejection. Alternatively, there is an immunological crossreactivity involved in the process. DNA sequence analyses have demonstrated not only that CMV encodes a molecule similar to the MHC class I antigen [2], but also that there is a sequence homology and immunological crossreactivity between some CMV-encoded molecules and the HLA-DR β-chain [7]. Some clinical evidence of the association between persisting CMV infection in the hepatocytes and chronic liver allograft rejection has been demonstrated [1]. The previous observation, that DR match is an important factor in the development of VBDS in association with CMV infection [16], could not be confirmed in our study.

The other cause of inflammation in the transplant was the alloresponse in most grafts. The frequency and severity of acute rejections are considered to be major risk factors for chronic rejection [9]. Whether acute rejections or rejection treatments induced activation of viral infections could not be proven, although they frequently occurred in the same patients. It is well known that rejection treatments, especially with OKT3 or ATG, predispose the patient to viral complications. In our patients, most CMV infections occurred after antirejection treatment, but they were also seen without preceding antirejection therapy, and there was no clear causal association. Nor was there clear causal evidence that viral infections could trigger acute rejection. However, in all patients there was an early lymphoid response in the graft, caused either by a virus or by alloreaction or both, that could have induced the immune mechanisms required for the slow process of chronic rejection.

The inflammatory episodes of rejection and viral infections were both associated with graft dysfunction. Graft dysfunction was still evident at the end of the hospitalization and close monitoring of the patients. The diagnosis of chronic rejection was established in most patients a few months later, which supports the previous suggestion that acute rejections or viral infections are risk factors for chronic rejection [1, 9, 16].

The overall incidence of acute rejection in liver transplant recipients in Hannover is 25 %, and about 10 % of them eventually develop chronic rejection. The inci-

dence of symptomatic CMV infection is 15 %, and about 65 % of these patients develop chronic rejection. The number of patients with HCV or HBV who develop chronic rejection is, in both cases, below 5 %, which corresponds to the overall incidence of chronic rejection, and these viral infections are generally not considered risk factors for chronic rejection. Thus, CMV seems to be a greater risk factor for chronic rejection than acute rejection. Other viral infections, such as HCV, may be involved, but their role is most likely limited to individual cases.

A long cold ischemia time is among the suggested risk factors for chronic allograft rejection [9]. Vascular injury and endothelial cell damage during the ischemia time may modify the immunogenicity of the graft and predispose it to liver allograft rejection [10]. The evidence that there is an association between cold ischemia time and chronic liver rejection is rather limited [15], and systematic studies with large clinical materials should be performed to prove it. The cold ischemia time in our 12 patients varied considerably; in most cases it was over 10 h, and in two cases it even exceeded 20 h. Thus, one could also speculate that this risk factor had an influence on the later clinical course of the recipients.

The role of the original disease in the development of chronic rejection is not known. Autoimmune diseases may be associated with a special immunoresponse of the recipient and also play a role in the post transplant course of the patient. Posthepatitis cirrhosis was the reason for liver transplantation in most of these cases, but reactivations of the virus occurred only in one case of hepatitis C; no serological evidence of hepatitis B reactivation was seen, probably due to the prophylactic therapy given. Thus, a reactivation of the hepatitis virus was not usually the cause of the post-transplant viral infections, and CMV was the most common virus causing the inflammatory episode in the graft.

In this study, we were unable to prove a direct association between viral infection and chronic liver allograft rejection, but we could demonstrate early intragraft inflammatory events that were associated with graft dysfunction leading to chronic rejection. The inflammatory infiltration and immunological triggering that appear to be necessary for the pathogenesis of chronic rejection [9] were evident in every graft. Thus, acute rejection or viral infection, especially CMV infection, or both induced the process. CMV seemed to be an even greater risk factor than acute rejection. Proof that the slow process is triggered by virus-induced immunoresponse or by alloresponse would require the continuous intragraft follow-up of the immunological events, cytokines, growth factors, and other parameters until chronic changes appear. In clinical transplantation, this kind of prospective study would be extremely difficult, time-consuming and expensive to carry out. Confirmation may have to come from various animal models developed to study chronic allograft rejection.

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