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Relevance of a positive crossmatch in liver transplantation

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Abstract. We studied 27 liver transplants in 24 patients performed between November 1984 and January 1988. We investigated retrospectively the importance of donor reactive HLA class I and class II and of non-HLA antibodies for graft survival in these patients. In order to determine the specificity and class of the antibodies, we used monoclonal antibodies to HLA-A, -B, -C and DR and DQ antigens to block cytotoxicity of sera and the reagent dithiothreitol to characterize the immunoglobulin class. We found that humoral immunity to HLA antigens in liver-grafted patients, demonstrable as the presence of cytotoxic antibodies reactive with donor splenic T and/or B cells in the pretransplantation period, is associated with significantly lower graft survival as compared with patients without demonstrable preformed HLA antibodies ($P = 0.01$). In addition we found that a substantial proportion of patients had donor-reactive cytotoxic antibodies which were not HLA specific. Thus, our study shows that HLA immunity can influence liver allograft survival, and that it is useful to have patient cytotoxic antibodies characterized with regard to HLA reactivity prior to transplantation.

Key words: Liver transplantation, crossmatch – Crossmatch, liver transplantation – Positive crossmatch, liver transplantation

Major histocompatibility complex (MHC) incompatible allografts induce an immune response leading to rejection. Susceptibility to rejection varies for different tissues and organs. For example experimental skin grafts are rapidly rejected, while liver allografts are more resistant to allograft immunity [5].

Immunopathological studies of hyperacute kidney graft rejection show binding of antibodies. This is followed by sludging of RBCs, formation of microthrombi on the glomerular and peritubular capillary walls and necrosis of the graft. Therefore, kidney allografts are not performed if the patient's serum contains cytotoxic antibodies directed against donor lymphocytes, since it may result in hyperacute rejection [19, 35, 36].

HLA matching improves graft survival of kidney, heart and cornea transplants [3, 11, 26, 28] whereas, in liver transplantation, HLA compatibility, on the one hand, may reduce transplant rejection, and, on the other, may enhance other immunological mechanisms leading to allograft dysfunction, particularly in patients at risk of developing recurrent autoimmune diseases or infection [23]. In addition, liver-transplanted patients rarely experience hyperacute rejections even in the presence of preformed cytotoxic antibodies [14, 17, 31]. Blood group compatibility, however, seems to be of importance for graft survival [32]. There are several possible explanations for the relative resistance of liver transplants to hyperacute rejection, such as in vivo absorption of pre-existing antibodies by perioperative transfusions, the result of a plasma exchange effect by multiple perioperative transfusions or resistance of liver graft cells to the effects of cytotoxic antibodies.

Whether the ability to transplant cadaveric livers successfully, in spite of a positive T and/or B cell crossmatch as compared to other organ grafts, is due to the unique anatomic structure of the liver or to differences in the expression of HLA antigens is not known. Expression of class I and class II MHC antigens on normal liver vasculature is now more clearly defined. In normal liver tissue, HLA class I antigens are expressed on endothelial, reticuloendothelial and biliary epithelial cells [2, 12, 20], but not on hepatocytes. However, it has been shown that HLA class I antigens are detected on hepatocytes in patients with hepatic diseases [37]. In addition, hepatocytes express class I antigens during acute liver allograft rejection episodes [25, 29].

HLA class II antigens are constitutively expressed on Kupffer cells, but not on hepatocytes in normal liver tissue. However, they are detected on bile duct epithelial cells during rejection [9, 34].

Gordon et al. [15] and Iwatsuki et al. [17] have reported that the presence of preformed donor-reactive cytotoxic antibodies in liver-transplanted patients is neither associ-

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ated with hyperacute rejection nor decreased graft survival. Since these reports, little work has been done to elucidate the humoral aspects of graft destruction in liver-transplanted patients. Recently, however, hyperacute rejection of experimental liver grafts (in monkeys) due to preformed antibodies has been reported [16]. In addition, one clinical report suggested that humoral immunity is associated with an increased frequency of rejections after liver transplantation. Donaldson et al. found that the incidence of vanishing bile duct syndrome VBDS), which is believed to be a variant of chronic rejection, is more frequent in patients who form cytotoxic antibodies in the post-transplantation period [10]. Using T cells from a panel of 30 donors they found high titre donor-specific platelet-absorbable antibodies believed to be involved in the pathogenesis of bile duct damage. Results from another study indicated that patients with a positive crossmatch are more likely to develop VBDS than the crossmatch-negative group [4]. These results suggest that the presence of cytotoxic HLA class I antibodies prior to transplantation may be associated with decreased graft survival.

In view of the above findings, we investigated, retrospectively, the importance of HLA class I and class II, and of non-HLA antibodies for graft survival in patients undergoing liver transplantation. We made a careful study of the specificity and class distribution of the antibodies detected in serum samples obtained pre- and post-transplantation, and have tried to draw conclusions as to the nature of 'immunization' that is compatible with survival of allogenic liver transplants.

Materials and methods

Patients and clinical events

Retrospective crossmatch results were obtained from 27 liver transplants in 24 patients performed between November 1984 and January 1988. All crossmatches were performed using only donor spleen cells. The patients were aged 2–55 years with a mean of 36.4 years and, all underwent orthotopic liver transplantation according to the technique described by Starzl et al. [30, 33]. The indications for liver transplantation were malignant hepatomas (6), sclerosing cholangitis (6), primary biliary cirrhosis (2), chronic active hepatitis (4), metabolic diseases (2), biliary atresia (1), non-A-non-B-hepatitis (1), and retransplantation (5).

Indications for retransplantation were acute or chronic rejection (2), primary non-function of the graft (1) and vascular thrombosis (2). The follow-up period ranged from 2 weeks (in cases of early graft loss) to 24 months (in patients with good graft survival). Immunosuppression was maintained by cyclosporin A and prednisolone. Of the 24 patients, 23 were treated at least once for rejection episodes during the first postoperative month. Mean first day of anti-rejection treatment was day 5 postoperatively. In 11 instances, rejection was proved by biopsy. Thus, in the majority of cases the diagnosis was based on clinical and laboratory parameters. Rejection episodes were treated with bolus doses of 1 g Solu-Medrone followed by an increase of the prednisolone to 200 mg/day which was tapered down to 20 mg/day over 5 days. In cases of steroid-resistant rejection, polyclonal anti-thymocyte globulin (ATG) or OKT3 (monoclonal antibodies directed against CD3 marker on T cells) was used and azathioprine was added as immunosuppressant just before discontinuing antibody treatment. OKT3/ATG was not given unless rejection was verified by core biopsy.

Collection of serum samples

Serum samples for T and B cell crossmatch analyses were taken immediately preoperatively and, if found positive, repeated daily for 14 days postoperatively. Thereafter, and for those with negative preoperative crossmatches, serum was obtained at 3 weeks, 6 weeks, 3 months, 6 months and 1 year post-transplantation. In total, 247 serum samples were studied.

Cell fractionation

Separation of donor spleen cells (kept frozen in liquid nitrogen) into T and B lymphocytes has been described previously [18]. In brief, the non-T-cell fractions were obtained by depleting cells forming rosettes with 2-aminoethylisothiouronium bromide-treated sheep red blood cells (AET-SRBC) [27]. The interface containing the non-T-cell fraction was washed and the T cells were obtained by incubating the rosettes with human AB serum for 15 min at 37 °C to lyse the SRBC.

Microlymphocytotoxicity test

Briefly, 0.5 µl serum was reacted with 0.5 µl of both T and non-T cells ($3–4 \times 10^6$/ml), and incubated at 22 °C for 30 min followed by addition of 2 µl rabbit complement. Post-complement time was 1 h for T cells and 2 h for non-T cells. The cells were then stained with trypan blue and counted. A positive crossmatch was scored when there was lysis of more than 10% above background of a T- or non-T-cell population. For simplicity the non-T-cell population was called 'B-cell population'.

All positive donor reactive sera were then titrated. The same sera were also tested for antinuclear antibodies (ANA), IgM rheumatoid factor (RF), HBsAg and HBsAg antibodies.

Determination of immunoglobulin class

Dithiothreitol (DTT) was used to digest IgM antibodies [3]. Sera were reacted with 0.01 *M* DTT at 37 °C for 30 min, followed by the standard NIH crossmatch. IgM autoantibodies and IgG alloantibodies were used as controls.

Platelet absorption

A pool of 50 outdated platelets from normal healthy human donors was used. Platelets already separated from leukocytes and red cells were obtained from the blood bank at our centre. The platelets were centrifuged at 2500 rpm for 15 min, after which 0.5 ml of packed platelets were added to 0.5 ml of antisera. The mixture was incubated for 1 h at room temperature with shaking, then centrifuged at 2500 rpm for 15 min. The supernatant was then transferred to another tube containing 0.5 ml fresh packed platelets. Absorption was carried out in a similar fashion three more times. The absorbed sera were then retested using the donor's T and B cells for reactivity. No specific controls were used since this method is used routinely and is found to work well.

Preparation of $F(ab')_2$ fragments for the determination of HLA class I or II specificity of donor reactive antibodies

Two cytotoxic mouse anti-human MHC monoclonal antibodies were used. One was directed against a monomorphic determinant on all known HLA class I antigens (clone w6/32) (source Serotec

Table 1. Immunoglobulin class specificity of donor reactive antibodies in liver transplanted patients

	No. of patients	Reaction after DTT treatment
Group 1: pretransplant	3	+
	7	−
post-transplant	3	+
	7	−
Group 2: post-transplant	11	−

Chemicals), and the other against a monomorphic determinant present on all known DR antigens (clone L243) (Becton Dickinson). A third non-cytotoxic monoclonal antibody directed against a monomorphic determinant present on DQw1 and DQw3 antigens (Leu-10) (Becton Dickinson) was also used. The two cytotoxic monoclonal antibodies were digested with pepsin to obtain the $F(ab')_2$ fragments. Pepsin concentration used was 20 mg/100 mg of the IgG concentration. The isotype of the two monoclonal antibodies was IgG2a. The protein (IgG) was dialysed against an acetate buffer overnight and the solution changed once. Excess pepsin was added to the protein and incubated at 37 °C for approximately 18 h. The digested protein was then dialysed against phosphate buffered saline containing 0.02% Na-azide, and kept overnight. The solution was changed once. The $F(ab')_2$ fragments were eluted after passing through a protein A column to remove any undigested protein and the Fc fragments. The purified $F(ab')_2$ fragments were then concentrated and used in a saturated concentration in the inhibition assay.

Inhibition assay

Donor spleen cells (0.5 µl, 4–5 × 10^6 cells/ml) were incubated with 0.5 µl non-cytotoxic anti-class I/DR/DQ antibodies for 1 h at 22 °C in duplicate. The selected neat sera and doubling dilutions of the sera were then added and the crossmatch was performed according to the standard cytotoxocity assay as mentioned above. On each occasion, one or more known specific anti HLA-class I, -DR and -DQ sera were used to confirm successful inhibition of cytotoxicity.

Criterion for inhibition assay using $F(ab')_2$ and the non-cytotoxic monoclonal antibodies

The two monoclonal antibodies (w6/32 and L243) were first tested with 21 different sera directed at specific HLA-A, -B, -C and -DR antigens. Specific HLA sera used were directed against: A1, 2, 3, 9, 10 and 19; HLA-B5, 7, 8, 12, 15, 27 and 35; and HLA-DR1, 2, 3, 4, 5, 6, 7 and 8. Alloantisera specific for HLA-DQw1 (including splits DQw5 and 6) and DQw3 (including splits DQw7, w8 and w9) were used to detect blocking by Leu-10. These monoclonal antibodies have been shown formally not to react with DQw2 (Becton Dickinson). Based on these experiments, the criterion chosen for positive inhibition was that cytotoxicity should be completely removed or the titre reduced by at least two $\log^2$ dilution steps. Evaluation of the liver transplantation crossmatches was done using the above criteria.

Definition of HLA antibodies

We have defined a serum as containing HLA antibodies if the reactivity with T and/or B cells is inhibited by any of the above mentioned monoclonal antibodies to human HLA class I, -DR or -DQ.

Definition of non-HLA antibodies

A serum is defined as containing only non-HLA antibodies if the reactivity of the antibodies with T and/or B cells is not inhibited by any of the above mentioned monoclonal antibodies.

Results

None of 27 grafts in 24 patients was hyperacutely rejected.

According to the HLA-immunization status of patients, and their respective grafts, the following groups were identified:

Group 1, which consist of ten grafts with a positive donor reactive T- and/or B-cell crossmatch on the day of transplantation (current serum) as well as during the post-transplantation period. Some of the patients had a negative crossmatch during the early post-transplantation period, but the sera from all these patients were positive during follow-up.

Group 2, which included 11 grafts with a positive donor-reactive T- and/or B-cell crossmatch only in the post transplantation period.

Group 3, which consisted of six transplants, with no demonstrable antibodies, either pre- or post-transplantation.

Titres and immunoglobulin class of donor reactive antibodies in liver-transplanted patients

Group 1. Cytotoxicity with sera from three patients with a positive crossmatch in the pretransplantation period were not affected by DTT treatment indicating the presence of non-IgM (most likely IgG) antibodies (1:2–1:16). In the post-transplantation period, the reactivity of serum from one of these patients was not reduced by DTT (IgG antibodies) (1:8), while the sera from the other two patients were completely reduced by DTT during the first 2 weeks (IgM antibodies) (1:32), but were not reduced by DTT in sera collected thereafter, indicating the presence of IgG antibodies (1:64) (Table 1).

Sera from seven patients were reduced by DTT in the pretransplantation period (IgM antibodies) (1:2–1:37), and the seven sera were also reduced by DTT in the post-transplantation period (1:32–1:64).

Group 2. The sera of all patients with demonstrable antibodies only in the posttransplantation period were completely inhibited by DTT, indicating the presence of IgM antibodies only (Table 1) (1:64–1:512).

No correlation of immunoglobulin class of antibodies with graft survival or rejection was observed.

HLA specificity and titres of donor reactive antibodies

Group 1. Table 2 gives the complete characterization of cytotoxic antibodies detected in sera obtained immediately prior to transplantation. In brief, the sera of patient no. 7, who received two grafts, had antibodies which were inhibited by class I monoclonal antibodies only. The sera of

Table 2. Characterization of antibodies responsible for a positive crossmatch prior to transplantation in liver transplant patients of group 1

Patient no.	Graft no.	Ig class	Reactive with	Reactivity after platelet absorptions	Specificity	Graft outcome
1	I	IgG	T + B (1:16)	–	Class I + DQ	Graft lost after 2 months
2	I	IgM	T + B (1:16)	–	Non-HLA + Class I (reduced by 25%)	Success
3	II	IgM	B (1:8)	–	Class I	Graft lost after 10 days
3	III	IgM	T + B (1:64)	–	Class I	Graft lost after 1 week
4	II	IgG	B (1:16)	+	DQ + DR	Graft lost after 15 days
5	I	IgM	B (1:8)	+	DQ	Graft lost after 1 month
6	I	IgM	B (1:64)	+	Non-HLA	Patient died with functioning graft
7	II	IgM	B (1:256)	+	Non-HLA	Success
8	I	IgM	B (1:512)	+	Non-HLA	Success
9	I	IgG	T + B (1:64)	–	Non-HLA	Success

patients nos. 1 and 2, in addition to class I antibodies, had DQ and non-HLA antibodies, respectively. The sera of patients nos. 6 and 8 had antibodies which were inhibited by class II monoclonal antibodies only, and patients nos. 3, 4, 5 and 9 had antibodies which were not inhibited either by class I or class II monoclonal antibodies (non-HLA).

A majority of patients (eight) had non-HLA antibodies in the post-transplantation period. The sera of four patients who had HLA antibodies in the pretransplantation period had non-HLA antibodies in the post-transplantation period. Only the sera of two patients (nos. 6 and 7) who had HLA antibodies in the pretransplantation period also had detectable class I and class II antibodies, respectively, after transplantation.

Group 2. The sera from most patients (six) who developed antibodies only in the posttransplantation period had non-HLA antibodies reactive with T and/or B cells (1:128–1:512). Sera from four patients reactive with T and/or B cells (1:16–1:32) had class I antibodies and sera from one patient with antibodies reactive with B cells only (1:16) had DQ antibodies (data not shown).

Platelet absorptions

As seen from Table 2, in the pretransplantation period, platelet absorptions in most cases correlated with inhibition assays, except for a few discrepancies. In some instances, extra specificities (class II and non-HLA) were detected only by the inhibition assays. In one instance, non-HLA antibodies were absorbed by platelets. In patients who developed antibodies only in the post-transplantation period, platelet absorptions did not correlate with inhibition assays. Again, certain sera with class I antibodies (detectable only by inhibition assay) were not platelet absorbable, and some sera with non-HLA antibodies were absorbed by platelets. The reasons for these discrepancies is discussed below.

Overall graft and patient survival

The overall actual graft and patient survival at 1 year was 63% and 71% respectively. Comparing the 1-year graft survival betwen the three groups we found that 4/10, 8/11 and 5/6 grafts survived in group 1, 2 and 3, respectively (Fig. 1).

Graft survival in patients with HLA versus non-HLA antibodies

Group 1. Of the six patients who had HLA antibodies in the pretransplantation period, only one graft survived for 1 year. In comparison, the grafts of three patients out of

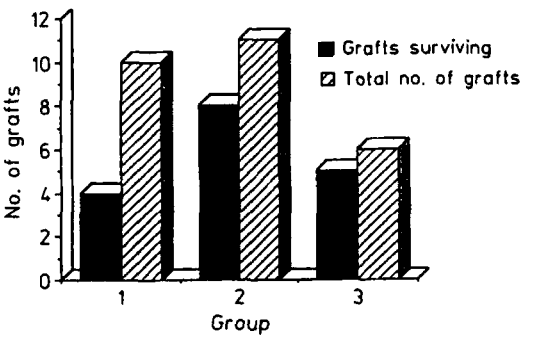

Fig. 1. One-year graft survival in patients with donor-specific antibodies to T and/or B cells pre-and post-transplantation (*group 1*), only during the post-transplantation period (*group 2*), or no demonstrable antibodies (*group 3*)

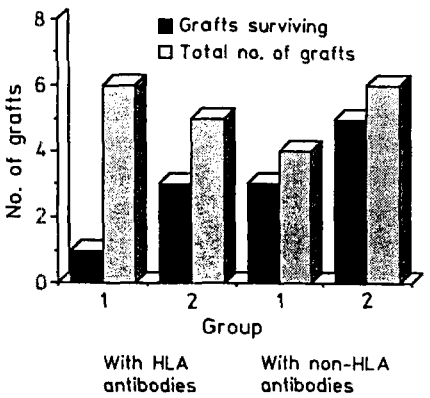

Fig. 2. One-year graft survival in patients *with HLA antibodies or non-HLA antibodies* in *groups 1* and *2*

four with non-HLA antibodies, were surviving at the end of 1 year.

Group 2. In patients who only developed donor reactive HLA specific antibodies after transplantation, three out of five grafts survived for 1 year, while in those with non-HLA antibodies, five out of six grafts survived (Fig. 2).

When we compared 1-year graft survival in patients with HLA antibodies in the pretransplantation period with graft survival in patients in whom no HLA antibodies were detected, we found that 1/6 and 16/21 grafts survived respectively ($P = 0.01$) (Fig. 3). In addition, in patients with HLA antibodies in the pre- and post-transplantation period or in only the post-transplantation period (groups 1 + 2), the overall graft survival was 4/11, which was of borderline significance as compared with patients with non-HLA antibodies, 8/10 ($P = 0.05$) (Fischer's exact test).

Non-HLA antibodies in liver transplanted patients

In order to elucidate the specificity of non-HLA antibodies demonstrable on donor splenic lymphocytes, we decided to check the presence of autoreactive antibodies with the following results.

Group 1. The serum from one patient in the pretransplantation period had antibodies reactive with the patient's own lymphocytes, indicating the presence of autoreactive antibodies. This was a patient with chronic active hepatitis. Unfortunately, autologous peripheral blood lymphocytes were only available from five patients, and when tested for the presence of autoantibodies using the peripheral blood lymphocytes they were found to be negative prior to transplantation.

Group 2. Autologous blood lymphocytes were obtained from 8/11 patients who had no demonstrable autoantibodies when tested in the post-transplantation period.

Infections

Most of the patients suffered at least once from either bacterial, fungal or viral infections during the follow-up period. Since many infections are also known to lead polyclonally to antibody production [18], we decided to characterize these antibodies. During infections, patients in both groups 1 and 2 had high titre donor-reactive DTT-reduced antibodies, which were not inhibited by either w6/32, or L243 Leu-10 (i.e. neither class I nor -DR nor -DQ antibodies) during infections. The same sera were also tested for ANA, RF, HBsAg and HBsAg antibodies. Only one patient was positive for HbsAg in the post-transplantation period, and the rest were all negative for ANA, RF and HBsAg antibodies.

Two case reports

Since this was a retrospective study, we found that a number of patients had undergone a transplant in the presence of a pretransplantation positive crossmatch. We therefore decided to study the sera of two such patients who had received multiple grafts, and from whom serum samples were collected every day post-transplantation for at least 2 weeks, and thereafter every week depending on the survival of each graft, in order to investigate whether the pattern of antibody production (including immunoglobulin class and specificity) in the post-transplantation period was predictive of rejection episodes or decreased graft survival.

Patient no. 4. (Table 1). A 49-year-old white male, who suffered from sclerosing cholangitis, underwent two liver transplantations. Prior to receiving the first graft, T- and B-cell crossmatches performed retrospectively with serum obtained on the day of transplantation were negative. However, 5 days posttransplantation, the patient developed donor-reactive DTT-insensitive DR-specific antibodies as well as non-HLA antibodies with titres of 1:8. He was treated for a biopsy-proven rejection with methylprednisolone 1 week post-transplantation. However, anti-rejection treatment was not successful and he lost his graft 10 days later (Fig. 4 a). Pathological examination of the removed graft showed portal vein thrombosis.

Only B-cell crossmatch was positive on the day of the second liver transplantation and the antibodies were characterized as a mixture of DQ- and DR-specific DTT-insensitive antibodies with titres of 1:16. Serum samples

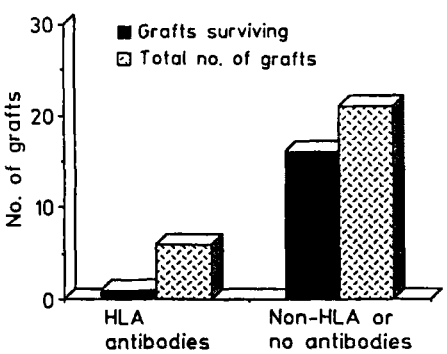

Fig. 3. One-year graft survival in patients with *HLA* and in patients with *non-HLA* or no demonstrable antibodies

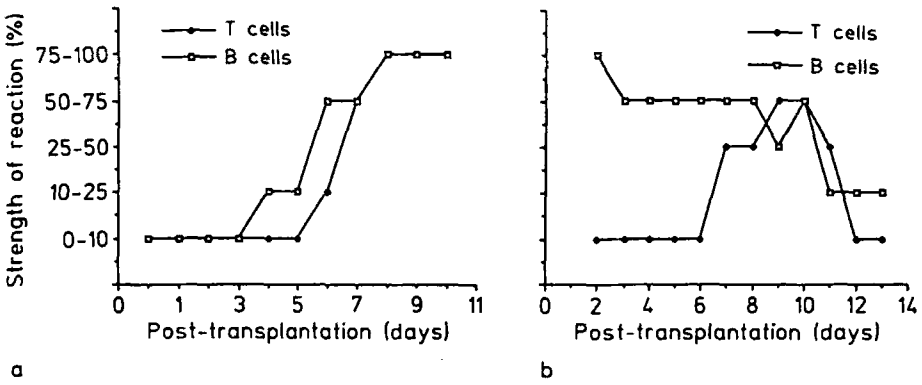

Fig. 4a, b. T and B cell donor reactivity of serum from patient no. 6. **a** Related to the first transplant. **b** Related to the second transplant

obtained at 1 week and thereafter every day post-transplantation showed the presence of DR and non-HLA antibodies. The patient died 15 days later from septicaemia (Fig. 4b).

Patient no. 3. (Table 1). A 40-year-old white female with sclerosing cholangitis received three grafts. A negative donor-specific T- and B-cell crossmatch was obtained using serum obtained immediately prior to the first transplant. However, 10 days post-transplantation the patient developed donor-reactive B-cell antibodies, which were characterized as IgM class I, with titres of 1:256. The patient experienced five rejection episodes which were treated with methylprednisolone. Class I antibodies were detected in various serum samples obtained until the end of 1 year, with titres of 1:32–1:512. The patient's first graft was lost in chronic rejection after 18 months. However, 3 months prior to graft loss, no donor-reactive antibodies were detected (Fig. 5a).

IgM class I donor-reactive antibodies to B cells (1:8) were detected in a serum sample obtained immediately prior to the second graft. Serum samples taken 1 day post-transplantation had non-HLA antibodies. However, the day-4 serum sample had IgM class I antibodies, which were detected in all the subsequent serum samples obtained post-transplantation with titres of 1:32–1:512. The second graft was lost due to acute rejection after 10 days (Fig. 5b).

Serum samples taken on the day immediately prior to the third graft had antibodies reactive with donor T and B cells which were found to be IgM class I (1:64). However, none of the serum samples obtained post-transplantation had class I or II antibodies. All positive T and B cell donor-reactive antibodies post-transplantation were due to non-HLA antibodies (Fig. 5c). The patient died due to multiple organ failure associated with a primary non-functioning graft.

Discussion

Our data suggest that the presence of preformed donor reactive HLA antibodies is associated with a decreased survival of liver grafts, but not with hyperacute rejection. One-year liver graft survival in patients with preformed donor reactive antibodies was not significantly different from those without demonstrable antibodies pre- and/or post-transplantation. This finding is similar to other reports [2, 14, 15, 17, 31, 32]. However, when our data are divided into those caused by HLA antibodies and those lacking HLA reactivity, an entirely different picture emerges. The group with donor reactive cytotoxic non-HLA specific antibodies had a higher success rate as compared with the relatively low graft survival of the HLA-immunized group. In previously published reports, no complete characterization of the antibodies was carried out. The use of the inhibition assay helped to differentiate the relevant HLA antibodies from the unimportant non-HLA reactivities.

In contrast to the relatively well studied reports on the T-cell destruction of liver allografts [7, 13, 21, 22], the role

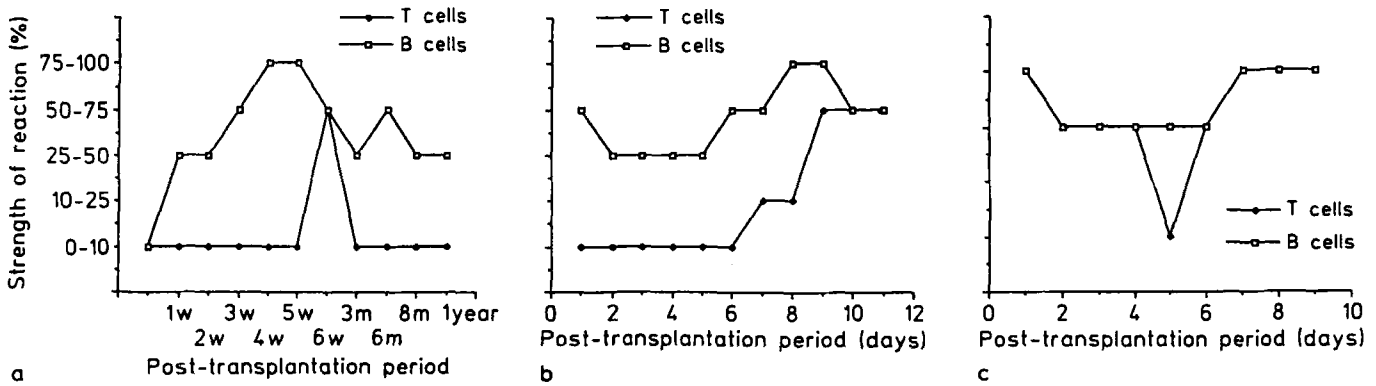

Fig. 5. Donor reactive antibodies ot T and B cells in the post-transplantation periods in patient no. 7 for **a** the first, **b** the second and **c** the third transplants

of antibodies is less well elucidated. Only a few studies have appeared since reports from the Pittsburgh group claimed that they found no adverse effect of preformed antibodies on liver graft survival [14, 17, 31]. Since then, the pretransplantation state of humoral sensitization in liver transplant patients has received very little attention. However, the recent study by Donaldson et al., in which they found high-titred donor-specific antibodies to class I antigens in patients with VBD syndrome but none in those without, implicates the role of humoral antibodies in liver allograft destruction [10]. Batts et al. have reported that, in their study, 5/6 patients with biopsy proven VBDS had positive crossmatches. They state that the risk of developing VBDS was significantly higher in patients with a positive crossmatch as compared with those with a negative crossmatch [4].

In the present study it was difficult to demonstrate a direct association of preformed antibodies with rejections, this could perhaps be due to the difficulty in distinguishing the various and combined effects of other destructive factors including ischaemia, sepsis, viral hepatitis and drug toxicity.

Reactions after platelet absorptions did not always correlate with the actual specificity of the antibodies present in the post-transplantation sera. It was often difficult to determine the precise specificity of the antibody based only on results obtained after platelet absorptions. One reason could be the complicated post-transplantation clinical scene, including perioperative transfusions, immunosuppression (ATG, OKT3 etc.), making it difficult to distinguish HLA class II and non-HLA antibodies after platelet absorptions. Another reason could be that a pool of 50 platelets may not represent all HLA-A, -B, -C specificities, especially those with a very low frequency. Certain class I specificities such as HLA-C antigens, B8, B12, etc. are not well expressed on platelets [1, 8]. In addition platelets also have receptors for IgG (FcγRII) and therefore may absorb immune complexes. Thus, platelet absorptions may give false-positive or false-negative results.

Some of the patients with non-HLA antibodies in the post-transplantation period had received rabbit anti-thymocyte globulin. It is, therefore, possible that these antibodies could be responsible for the positive reactions [24]. On the other hand, five patients who had non-HLA antibodies in the post-transplantation period had not received ATG. Another cause for the presence of non-HLA antibodies could be polyclonal B-cell activation directly by viruses or bacterial products or indirectly through inflammatory mediators during infections. A majority of patients suffered from various viral, fungal and bacterial infections after transplantation. In addition, it is likely that these patients had autoantibodies that were not detected using peripheral blood lymphocytes, since they are known to be less sensitive to cytotoxic antibodies than spleen lymphocytes. Furthermore, it is not possible to type for class II antigens using peripheral blood lymphocytes from patients with end-stage liver diseases, many reactions giving false-negative results. Thus, the lower sensitivity of peripheral blood lymphocytes to cytotoxic antibodies is again demonstrated.

No correlation was found between antibodies and rejections or decreased graft survival in the post-transplantation period. Thus immunological monitoring of sera from liver-transplanted patients may not be of much value.

With the improved preservation of the livers achieved during recent years, cold ischaemia can be up to 20 h. This enables time to perform crossmatches. Using more sensitive and rapid techniques such as the immunomagnetic method [38], combined with inhibition assays such as that described by Chapman et al. [6], it is possible to detect antibodies which are clinically relevant. This study suggests that HLA antibodies present in crossmatch sera of liver-transplanted patients are clinically relevant, and therefore transplantation in such cases should preferably be performed with a crossmatch-negative donor. On the other hand, patients with a positive crossmatch caused by non-HLA antibodies may be transplanted successfully. Our material is relatively limited, and a larger study, with a greater number of patients, is required. Similar studies from other laboratories will be necessary to elucidate the significance of preformed HLA antibodies in the outcome of liver allografts.

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References

1. Aster RH, Szarkowski N, Liedert M, Duquesnoy RJ (1977) Expression of HLA-B12, HLA-B8,w4 and w6 on platelets. Transplant Proc 9: 1695–1696
2. Barbatis C, Woods J, Morton JA, Fleming KA, McMichael A, McGee JO (1981) Immunohistochemical analysis of HLA (A, B, C) antigens in liver disease using a monoclonal antibody. Gut 22: 985–991
3. Batchelor JR, Casey TA, Werb A, Gibbs DC, Prasad SS, Llyoyd DF, James A (1976) HLA and corneal grafting. Lancet I: 551–554
4. Batts KP, Moore SB, Perkins JD, Wiesner RH, Grambsch PM, Krom RAF (1988) Influence of positive lymphocyte crossmatch and HLA mismatching on vanishing bile duct syndrome in human liver allografts. Transplantation 45: 376–379
5. Calne RY, White HJO, Joffa DE, Maginn RR, Binns RM, Samuel JR, Molina VP (1987) Observations of orthotopic transplantation in pig. Br Med J 2: 478–480
6. Chapman JR, Taylor CJ, Ting A, Morris PJ (1986) Immunoglobulin class and specificity of antibodies causing positive T cell cross-matches: relationship with renal transplant outcome. Transplantation 42: 608–613
7. Colson YL, Markus BH, Zeevi A, Duquesnoy RJ (1988) Interactions between endothelial cells and alloreactive T cells involved in allograft immunity. Transplant Proc 20: 273–275
8. Curtoni ES, Borelli I, Carbonara AO, Diotallevi P, Olivetti E, Neri TM, Savi M (1979) Serological and genetic aspects of HLA-DR antigen study. Transplant Proc 11: 1317–1318
9. Demetris AJ, Lasky S, Thiel DM van, Starzl TE, Whiteside T (1985) Induction of DR/IA antigens in human liver allografts: an immunocytochemical and clinicopathological analysis of twenty failed grafts. Transplantation 40: 504–509
10. Donaldson PT, Alexander GJM, O'Grady JG, Neuberger J, Portmann B, Thick M, Davis M, Calne RY (1987) Evidence for an immune response to HLA class I antigens in the vanishing bile duct syndrome after liver transplantation. Lancet I: 945–948

11. Festenstein H, Doyle P, Holmes J (1986) Long-term follow up in London group recipients of renal allografts. The influence of HLA-matching on transplant outcome. N Engl J Med 314: 7–14
12. Fleming KA, McMichael A, Morton JA, Woods J, McGee JO (1981) Distribution of HLA class I antigens in normal human tissue and in mammary cancer. J Clin Pathol 34: 779–784
13. Fung JJ, Markus BH, Zerbe TR, Duquesnoy RJ (1986) Dynamics of allospecific T lymphocyte infiltration in vascularized human allografts. Immunol Res 5: 149–163
14. Gordon RD, Fung JJ, Markus BM, Fox I, Iwatsuki S, Esquivel CO, Tzakis A, Todo S, Starzl TE (1986) The antibody crossmatch in liver transplantation. Surgery 100: 705–715
15. Gordon RD, Fung JJ, Iwatsuki S, Duquesnoy RJ, Starzl TE (1988) Immunological factors influencing liver graft survival. Gastroenterol Clin North Am 17: 53–59
16. Gubernatis A, Lauchart W, Jonker M, Steinhoff G, Bornschever A, Neuhaus P, Es AA van, Kemnitz J, Wonigeit K, Pichlmayr R (1987) Signs of hyperacute rejection of liver grafts in rhesus monkeys after donor-specific presensitization. Transplant Proc 19: 1082–1083
17. Iwatsuki S, Ratsin BS, Shaw BW Jr (1984) Liver transplantation against T cell-positive warm crossmatches. Transplant Proc 16: 1427–1429
18. Karuppan S, Lindholm A, Möller E (1988) Pre- and posttransplant sensitization in cyclosporin A-treated patients. Clin Transplant 2: 245–251
19. Kissmeyer-Nielsen F, Olsen S, Petersen VP, Fjeldborg O (1966) Hyperacute rejection of kidney allografts, associated with preexisting humoral antibodies against donor cells. Lancet II: 662–665
20. Lautenschläger I, Häyry P (1981) Expression of the major histocompatibility complex antigens on different liver cellular components in rat and man. Scand J Immunol 14: 421–426
21. Markus BH, Colson YL, Fung JJ, Zeevi A, Duquesnoy RJ (1988) HLA antigen expression on cultured human arterial endothelial cells. Tissue Antigens 32: 241–253
22. Markus BH, Demetris AJ, Fung JJ, Saidman S, Zeevi A, Starzl TE, Duquesnoy RJ (1988) Allospecificity of liver allograft-derived lymphocytes and correlation with clinicopathologic findings. Transplant Proc 20: 219–222
23. Markus BH, Duquesnoy RJ, Gordon RD, Fung JJ, Vanek M, Klintmalm G, Bryan C, Thiel DV, Starzl TE (1988) Histocompatibility and liver transplant outcome. Does HLA exert a dualistic effect? Transplantation 46: 372–377
24. Martin S, Brenchley PE, O'Donoghue DJ, Dyer PA, Mallick NP, Johnson RW (1988) The identification of allo and autolymphocytotoxic antibodies in serum, in the presence of rabbit thymocyte globulin. Tissue Antigens 31: 26–32
25. Nagafuchi Y, Hobbs KEF, Thomas HC, Scheuer PJ (1985) Expression of beta-2 microglobulin in hepatocytes after liver transplantation. Lancet I: 551–554
26. Opelz G (1985) Correlation of HLA matching with kidney graft survival in patients with or without cyclosporin treatment. Transplantation 40: 240–243
27. Pellegrino MA, Feroone S, Theofilopoulos AN (1976) Isolation of human T and B-lymphocytes by rosette formation with 2-aminoethyl isothiouronium bromide (AET)-treated sheep red blood cells and with monkey red blood cells. J Immunol Methods 11: 273–279
28. Sanfilippo F, MacQueen M, Vaughn W, Foulks G (1986) Reduced graft rejection with good HLA A & B matching in high-risk corneal transplantation. N Engl J Med 315: 29–35
29. So SKS, Platt JL, Ascher NL, Snover DC (1987) Increased expression of class I major histocompatibility complex antigens on hepatocytes in rejecting human liver allografts. Transplantation 43: 79–85
30. Starzl TE (with the assistance of Putnam CW) (1969) Experience in hepatic transplantation. WB Saunders, Philadelphia
31. Starzl TE, Ishikawa M, Putnam CW, Porter KA, Piache R, Husberg BS, Halgrimson CG, Schroter G (1974) Progress in and deterrents to orthotopic liver transplantation, with special reference to survival, resistance to hyperacute rejection and biliary duct reconstitution. Transplant Proc 6: 129–138
32. Starzl TE, Koep LJ, Halgrimson CG, Hood J, Schroter GPJ, Porter KA, Weil R III (1979) Fifteen years of clinical liver transplantation. Gastroenterology 77: 375–388
33. Starzl TE, Iwatsuki S, Esquivel CO, Todo S, Kam I, Lynch S, Gordon RD, Shaw BW Jr (1985) Refinements in the surgical technique of liver transplantation. Semin Liver Dis 5: 349–356
34. Takacs L, Szende B, Monostori E, Rot A, Lapis K, Szecseny A, Ando I (1983) Expression of HLA-DR antigens on bile duct cells of rejected liver transplants. Lancet II: 1500
35. Terasaki PI, Bernoco D, Park MS, Turk G, Iwaki Y (1978) Microdroplet testing for HLA-A, B, C & D antigens (The Phillip Levine Award Lecture). Am J Clin Pathol 69: 103–120
36. Terasaki PI, Marchioro TL, Starzl TE (1985) Serotyping of human lymphocyte antigens: preliminary trials on long term kidney homograft survivors. In: Russell PS, Winn MJ, Amos DB (eds) Histocompatibility testing. National Academy of Sciences, National Research Council, Washington DC, pp 83–96
37. Thung SN, Schaffner F, Gerber MA (1988) Expression of HLA antigens on hepatocytes in liver disease. Transplant Proc 20: 722–723
38. Vartdal F, Gaudernack G, Funderud S, Brattie A, Lea T, Ugerstad S, Thorsby E (1986) HLA class I and II typing using cells positively selected from blood by immunomagnetic isolation – a fast and reliable technique. Tissue Antigens 28: 301–312