Balance between herpes viruses and immunosuppression after lung transplantation

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Chapter 6

Quantitative Epstein-Barr virus (EBV) Serology in Lung Transplant Recipients with primary EBV Infection and/or Post Transplant Lymphoproliferative Disease

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Journal of Medical Virology, 2003:69:258-266

Abstract

The Epstein-Barr virus (EBV)-specific antibody response was studied in lung transplant patients to assess their value in the diagnosis and prognosis of post-transplant lymphoproliferative disease. Recently developed synthetic peptides representing Epstein-Barr nuclear antigen-1 (EBNA-1), diffuse early antigen (EA(D)), and virus capsid antigen (VCA) were studied in a semiquantitative enzyme-linked immunosorbent assay (ELISA) to study antibody patterns in 12 seronegative lung transplant patients, of whom four developed a post-transplant lymphoproliferative disease, and seven seropositive lung transplant patients, all of whom developed a post-transplant lymphoproliferative disease. Immunoblot technique was used as a control. All 12 EBV-seronegative patients had a very limited antibody response that was restricted mainly to VCA antibodies. EA(D) antibodies became detectable in only two patients. Antibody response never preceded clinical diagnosis of post-transplant lymphoproliferative disease in the four EBV-seronegative patients who developed post-transplant lymphoproliferative disease. In the seven seropositive lung transplant patients with post-transplant lymphoproliferative disease, we found a rise in antibody titre in only two patients. Immunoblot analysis confirmed the serological results. In conclusion, EBV-specific antibody patterns after lung transplantation are highly restricted and variable and of limited value for the diagnosis or prognosis of post-transplant lymphoproliferative disease.

Introduction

Epstein-Barr virus (EBV)-induced post-transplant lymphoproliferative disease is a serious complication after solid-organ transplantation. EBV-seronegative status at transplantation is the main risk factor, and lung transplantation patients, most likely due to the high level of immune suppression, are particularly at risk. In this study, 4-10% of lung transplantation patients develop post-transplant lymphoproliferative disease [Walker et al., 1995a,b; Aris et al., 1996].

Traditionally, cellular immune responses, and in particular EBV-specific cytotoxic T-lymphocytes (CTLs), are considered most important for EBV control in the immune compromised host [Murray et al., 1992]. The role of antibodies in prevention of post-transplant lymphoproliferative disease is unclear. Although there are some suggestions that EBV-specific antibodies can prevent EBV-induced lymphoproliferative disease in the severe combined immune-deficient mouse model [Abedi et al., 1997; Nadal et al., 1997], suggesting that the humoral immune response could be important in the prevention of post-transplant lymphoproliferative disease.

EBV-specific serology is usually carried out using fixed whole cell preparations of EBV-transformed cells and immune fluorescence. Several patterns have been described for EBV serology in the immune compromised host and before diagnosis of post-transplant lymphoproliferative disease. A reduced titer of EBNA antibodies was considered to be associated with immune suppression and the risk of post-transplant lymphoproliferative disease [Preiksaitis et al., 1992; Riddler et al., 1994]. A sharp decrease before the diagnosis of post-transplant lymphoproliferative disease has also been described [McKnight et al., 1994]. Other studies reported an association with a rising EBV-IgG titer and post-transplant lymphoproliferative disease [Rogers et al., 1997], or an elevated antibody titre against the viral capsid antigen [McKnight et al., 1994]. Thus, it is unclear which serological pattern is predictive of post-transplant lymphoproliferative disease.

To clarify further the role of EBV-specific serology in the diagnosis, prognosis, and possible prevention of post-transplant lymphoproliferative disease, a semiquantitative enzyme-linked immunosorbent assay (ELISA) was used to measure IgM and IgG responses to three newly defined synthetic peptides representing combined immunodominant epitopes from Epstein-Barr nuclear antigen-1 (EBNA-1), diffuse early antigen (EA(D)), and the virus capsid antigen (VCA) [van Grunsven et al., 1994; Middeldorp et al., 1997]. In addition, to

analyze the molecular diversity underlying the anti-EBV antibody response, the spectrum of EBV antigens recognized by antibodies in (selected) patient sera was determined using immunoblot analysis.

The main goals were to study the humoral immune response during EBV infection under immunosuppression and during the development of post-transplant lymphoproliferative disease, and to study whether the antibody patterns were associated with and predictive for the development of post-transplant lymphoproliferative disease. For this purpose, lung transplant recipients with particular risk of EBV-related disease, i.e., seromismatched recipients and/or recipients with post-transplant lymphoproliferative disease, were studied longitudinally after lung transplantation.

Patients and Methods

Patients and treatment

Between November 1990 and May 1998, 118 lung transplantations were performed at the University Hospital Groningen (116 primary lung transplantations and 2 re-transplantations). Twelve patients were EBV seronegative before transplantation. One of these patients died 23 days after lung transplantation and was excluded from serological evaluation. All but one (Table 1, patient 12) received a transplant of a confirmed EBV-seropositive donor. Immunosuppression included rabbit-anti-thymocyte globulin 3 mg/kg (Thymoglobulin7, Merieux) 2-5 times postoperatively, Cyclosporine A (Neoral, Novartis, Switzerland) (dose adjusted to whole blood trough levels of 400 µg/L, within 3 weeks tapering to trough levels of 150 p g/L), azathioprine 1.53.0 mg/kg/day, and prednisolone 125 mg tid the first day, 0.2 mg/kg/day from day 2 until 6 months and 0.1 mg/kg/ day thereafter. Rejection episodes were diagnosed clinically and/or by histology of transbronchial biopsies. Acute rejection was treated with pulse therapy methylprednisolone (500-1,000 mg iv daily for 3 days). Steroid-resistant rejection was treated with horse-anti-thymocyte globulin.

Bronchiolitis obliterans syndrome was defined according to the criteria of the International Society for Heart and Lung Transplantation, with a grading ranging from optimal transplant function (grade 0) to severe dysfunction (grade 3).

***Table 1**: Patient characteristics*

	Age at Ltx		**PTLD**	**PTLD**	
Patient no.	(yr)/Sex	Diagnosis	localization	treatment	Outcome
1	44/M	EMP α1	Lungs	IS.red., ACV	CR BOS grade 3a (73 mo. after Ltx)
1 (relapse)			Sigmoid	IS.red., ACV, surgery	CR BOS grade 3a (73 mo. after Ltx)
2	9/M	PPH	Lungs	IS.red., ACV	CR BOS grade 0 (45 mo. after Ltx)
3	22/M	CF	Lungs	IS.red., V-ACV	PR, died of BOS grade 3a (14 mo. after Ltx)
4	39/M	BE	Nasopharynx	IS.red., V-ACV, surgery, chemotherapy	CR, died of disseminated myeloma (74 (mo. after Ltx)
5	48/M	EMP	Nasopharynx	-	-
6	22/M	LCH	-	-	Died of pulmonary hemorrhage (2 mo after Ltx)
7	25/M	EMP	-	-	-
8	22/M	CF	-	-	-
9	22/M	PPH	-	-	PGF, died (23 days alter Ltx)
10**	25/M	CF	-	-	-
11***	33/M	EMP α1	-	-	-
12***	13/F	CF	-	-	BOS grade 0 (10 mo after re-Ltx (because of BOS grade 3b) 13 mo after first Ltx)
13	43/M	EMP α1	Liver	IS.red./stop. ACV, chemotherapy	CR died of heart/renal failure (61 mo after Ltx), BOS grade 0a
14	20/M	CF	Rectum	No	Died of BOS grade 3b (40 mo after Ltx, 2 mo after PTLD)
15	56/11	EMP	Lungs, heart, ileum	No	BOS grade 3a, died of intestinal perforation (12 mo after Ltx) due to PTLD
16	44/F	CF	Cerebrum	IS.red., intrathecal chemotherapy	Died of PTLD (12 days after diagnosis, 6 mo after Ltx)
17	37/F	PPH	Lungs	IS.red., ACV, chemotherapy	Died of sepsis after 4th course of chemotherapy (10 mo alter Ltx)
18	58/M	EMP α1	Pleural cavity	IS.red., ACV	CR died of BOS grade 3b (31 mo after Ltx)
19	46/F	BE	Meckel's diverticulum	Surgery, IS.red., V-ACV	CR BOS grade 0 (56 mo alter Ltx)

PTLD, post-transplant lymphoproliferative disease; EMP α_1, emphysema due to a1-antitrypsin deficiency; PPH, primary pulmonary hypertension; CF.cystic fibrosis: BE. bronchietasies: EMP emphysema: LCH. Langerhans cell histiocytosis: IS.red.,reduction of immunosuppression; ACV. high-dose (800 mg five times daily) aciclovir; V-ACV, valaciclovir (1,000 mg tid); CR complete remission; PR, partial remission; BOS, bronchiolitis obliterans syndrome; PGF, primary graft failure Ltx, lung transplantation.
*Follow-up 10 weeks donor seropositive.
**Follow-up 54 weeks donor serostatus unknown.
***Follow-up 27 weeks donor seropositive.
****Follow-up 20 weeks donor serostatus unknown

Treatment of bronchiolitis obliterans syndrome was attempted with augmentation of immunosuppression (i.e., anti-thymocyte globulin during the initial years of our program, conversion from cyclosporine A to tacrolimus (Prograft, Fujisawa, Japan) during recent years). Standard antiviral prophylaxis was acyclovir 200 mg qd for 6 months. In the case of post-transplant lymphoproliferative disease, the dose of cyclosporine A was reduced to target trough levels of 75-100 µg/L, and acyclovir was increased to 800 mg, 5 doses per day.

Recently, valaciclovir (1,000 mg tid) was used instead of acyclovir. Two patients received additional chemotherapy (cyclophos-phamide doxorubicin vincristine prednisolone).

Pathology

The diagnosis of post-transplant lymphoproliferative disease was based on histological demonstration of a lymphoid proliferation. All samples were also stained with monoclonal antibodies against CD20/CD22, CD30, CD45 (DAKO, Glostrup, Denmark), and, in the case of suspected plasmacytoid differentiation, against CD138 (IQ products, Groningen, The Netherlands) with monoclonal antibodies against EBNA-2 (PE2 antibody; DAKO, Glostrup, Denmark), which was later confirmed by immunostaining against latent membrane protein-1 (OT21C antibody; Organon Teknika, Boxtel, The Netherlands) and RNA in situ hybridization (RISH) for Epstein-Barr encoded RNA-1,2 expression [Brink et al., 1997] (DAKO) (see Table 2). In one EBV-seronegative patient, no histology could be obtained and the diagnosis was based on radiological demonstrated masses in the transplanted lung and concomitant EBV seroconversion.

EBV serology

Sera from all patients were obtained twice weekly during hospitalization and at every outpatient visit and were stored at –20°C until use. From each patient, 16 serum samples (1 serum before transplantation, 15 sera before and after post-transplant lymphoproliferative disease) were examined simultaneously. A total of 12 seronegative recipients with or without post-transplant lymphoproliferative disease and seven seropositive recipients with post-transplant lymphoproliferative disease were studied. EBV-specific IgG and IgM antibodies were determined by semiquantitative ELISA using newly defined synthetic peptides (kindly donated by Organon Teknika, Boxtel, The Netherlands) representing EBNA-1, EA(D), and VCA. These peptides are 60, 51, and 56 amino acids in size respectively and combined several immunodominant epitopes of the EBV proteins encoded by the $BKRF_1$, $BMRF_1$, and $BFRF_3$ open reading frames respectively [van Grunsven et al., 1994; Middeldorp et al., 1997].

The test procedure is as follows. Polystyrene micro-titre plates are coated during 48 hr at 4°C with the peptide solution at 1 lig/m1 in 0.1 M sodium carbonate (pH 9.6), subsequently blocked with bovine serum albumin (BSA, 3%) and sucrose (5%) in phosphate-buffered saline (PBS, 0.1 M sodium phosphate, pH 7.4, containing 0.9% NaC1), dried at room temperature and stored at 4°C until use. Plates are washed, and sera are diluted in incubation buffer containing 0.01 M Tris, 0.3 M NaC1, 0.05% Tween-20, and 2% BSA (pH 7.5).

Table 2: *Serology in EBV seronegative Ltx recipients*

Patient no.	Onset of PTLD (weeks after Ltx)	EBV seroconversion (weeks after Ltx)				
		IgM		IgG		
		EA(D)	VCA	EBNA-1	EA(D)	VCA
1	7	-	-	-	-	-
1 (relapse)	82	-	95	-	-	114
2	6	-	7	-	-	10
3	11	13	11	-	-	21
4	250	-	17	-	-	17
5	-	32	32	-	-	41
6	-	-	-	-	-	-*
7	-	-	78	-	-	-
8	-	-	10	-	-	27
9	-	†	†	†	†	†
10	-	-	-	-	-	-**
11	-	-	-	-	-	-***
12	-	-	-	-	-	-****

† patient died 23 days post transplantation
* follow-up 10 weeks, donor seropositive
** follow-up 54 weeks, donor serostatus unknown
*** follow-up 27 weeks, donor seropositive
**** follow-up 20 weeks, donor serostatus unknown

Sera are diluted 1:100, 1:200, 1:400, and 1:800 for IgG and IgM; 100 1.11 of each serum dilution is incubated in the plates on an ELISA shaker for 45 min at room temperature. Plates are washed and conjugates added: IgG conjugate, goat-anti-human IgG-peroxidaselabeled (De Beer, Diessen, The Netherlands), IgM conjugate, goat-anti-human IgM-peroxidase-labeled (Pasteur, Marnes la Coquettes, France). Plates with conjugates are incubated for 30 min at room temperature and are washed. Substrate, 100 µl of 0.3 g/L OPD in citrate-buffered solution (pH = 5.05) with 0.0002% H_20_2, is added and after 20 min 100 p,1 of 1 M H_2SO_4 is added to stop the reaction. Plates are read at 490 nm.

In every assay, four EBV-negative control sera are tested to determine cutoff values (mean + 3 x SD). As a standard for IgG anti-EBNA-1 and anti-VCA reactivity, serum from a healthy seropositive volunteer is used. Standard for IgG-anti-EA(D) was obtained from a chronic EBV patient with high anti-EA(D) titres and as a standard for IgM-anti-EA(D) and anti-VCA sera from a patient with infectious mononucleosis is used. The amount of antibody in patient serum is expressed as a percentage of the standard serum which is included in each plate as described earlier [van der Giessen et al., 1990].

Immunoblot assay

Immunoblot analysis was performed essentially as described by Middeldorp and Herbrink [1988] and van Grunsven et al. [1993]. Nuclear extracts of EA + VCAinduced HH514 cells are separated in 10% acrylamide gels and are transferred to nitrocellulose sheets (0.22F, Schleicher and Schull, s'Hertogenbosch, The Netherlands). The sheets are saturated in blocking buffer, i.e., PBS containing 5% horse serum (Gibco, Breda, The Netherlands), and 3% nonfat dry milk (Campina, Veghel, The Netherlands) and cut into 3-mm strips. Each strip is incubated with human serum diluted 1:100 in blocking buffer for 1 hr and is washed subsequently three times with PBS containing 0.05% Tween-20. A second antibody (peroxidase-labeled rabbit anti-human IgG, DAKO, Glostrup, Denmark) is incubated in blocking buffer as described above, and the strips are washed with PBS-Tween and PBS, twice each, followed by detection of bound peroxidase, using 4-chloronaphthol as the substrate. The reaction is stopped by washing with H_20; the strips are then air-dried.

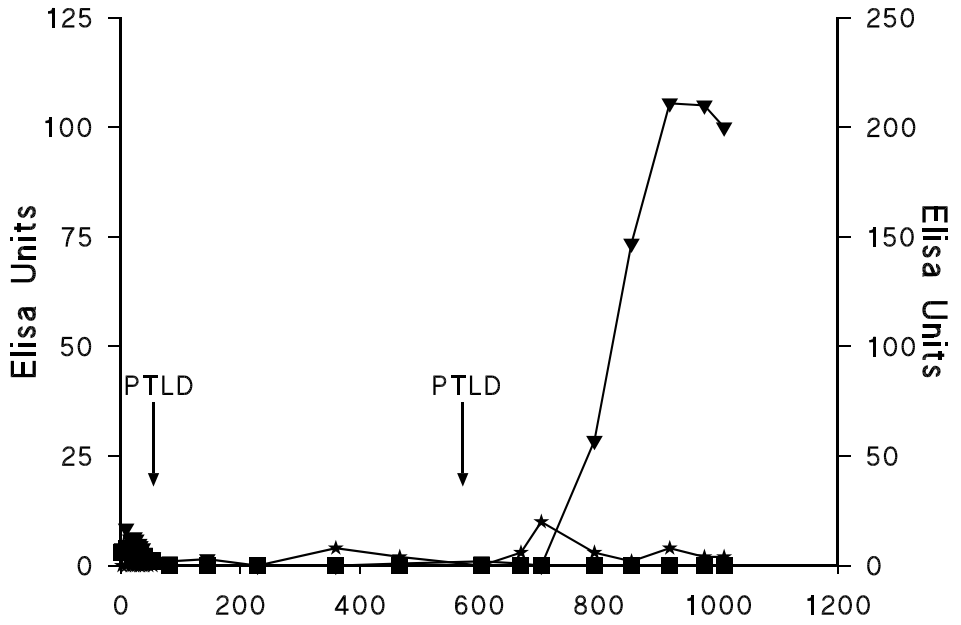
Results

Between 1990 and July 1998, 118 lung transplantations were carried out in 116 patients in our centre. Twelve patients were EBV seronegative and 102 patients were EBV seropositive before transplantation and the serostatus of two patients was undetermined (Table 1). Four of the 12 (33%) seronegative recipients versus 7 out of 103 (7%) seropositive recipients post-transplant lymphoproliferative disease *(P* 0.0034 , chi square). In post-transplant lymphoproliferative disease, EBER RISH and immunocytochemical staining for EBNA-2 and/or LMP1 (Table 3) demonstrated the presence of EBV. No EBV-negative lymphoproliferations were found.

First, anti-EBV antibody repertoires were analyzed. Of the 12 seronegative patients, most received a transplant from an EBV-seropositive donor (except patient 10 (Table 2)). Anti-VCA IgG and IgM class antibodies were most prominent, while anti-EA and anti-EBNA antibodies were an exception. Three patients developed an early post-transplant lymphoproliferative disease at 6, 7, and 11 weeks after transplantation (Table 2). No representative histology could be obtained from one patient. In the other two patients, a large B-cell blastoid lymphoproliferation was demonstrated. Two of these developed EBV antibodies at (patient 3) or shortly after (patient 2) diagnosis of post-transplant lymphoproliferative disease, including IgM anti-EA(D) in one. One (patient 1) failed to develop anti-EBV antibodies after the first episode of post-transplant

lymphoproliferative disease and developed a second post-transplant lymphoproliferative disease 18 months after lung transplantation in the sigmoid. Only after this episode did he develop a serological response consisting of IgM and IgG anti-VCA (Fig. 1). The fourth patient developed an EBV positive intranasal plasmacytoma 4,5 years after lung transplantation. He had suffered from chronic sinusitis for years.

***Figure 1**: Serologic pattern in lung transplant patient 1 with primary EBV infection and 2 episodes of PTLD. Data are expressed as Elisa Units (see Materials & Methods). IgM against VCA was detected at low levels > 600 days after Ltx and IgG against VCA (Y2 axis) became positive almost 800 days after Ltx. No IgG or IgM against EBNA-1 and EA(D) was found.*



He had developed IgM and IgG anti-VCA antibodies at 17 weeks after lung transplantation in high titre (Fig. 2) that had gradually decreased. No change in titre was observed shortly before the diagnosis of plasmacytoma. Of 8 patients who did not develop PTLD, one was excluded from serological evaluation due to early death, three developed serological signs of aprimary infection with a partial response consisting of exclusively IgM and IgG to VCA in two patients (patient 7 and 8) and additional IgM to EA(D) in one patient (patient 5). The other four patients remained seronegative during follow up of 10, 20, 27 and 54 weeks.

Seven of 102 (7%) EBV seropositive recipients developed PTLD at 4, 5, 6, 12, 32, 39 and 65 months after Ltx (Table 4). Limited and heterogeneous antibody responses could be demonstrated in 4 of 7 patients, in 2 subjects before the diagnosis of PTLD. No serological changes were seen in the remaining patients.

***Table 3**: Histology, immunostaining and EBV detection of PTLD's.*

Patient no.	Histology	Immunohistological staining	EBV detection
1	Large B-cell blastoid lymphoproliferation	ND	failed
1 (relapse)	large B-cell blastoid lymphoproliferation		LMP, EBER
2	not obtained	-	-
3	large B-cell blastoid lymphoproliferation	CD20/CD22 IgM, Lambda	EBER
4	plasmacytoma	CD138, IgA (CD20 neg)	EBER
13	large cell anaplastic B-cell lymphoma	CD20, CD30, CD45	EBER
14	large B-cell blastoid lymphoproliferation	ND	EBER
15	blastoid cells, some with plasmacytoid differentation	L26 (CD20)	LMP
16	large B-cell blastoid lymphoproliferation	L26 (CD20)	EBNA-2, LMP
17	large B-cell blastoid lymphoproliferation	CD20/22	LMP
18	large B-cell blastoid lymphoproliferation	CD20/22, CD21, mostly kappa, some lambda	LMP
19	monomorfous large cell B-cell lymphoma	CD20, CD45, partly CD30	EBER

EBV, Epstein-Barr virus; EBNA, Epstein-Barr nuclear antigen; LMP, Latent membrane protein.
Patients 1-4: EBV seronegative before Ltx. Patients 13-19: EBV seropositive before Ltx. Routine immunological staining was done for CD20/CD22 (B-cell), CD45, CD3 (T-cell) and, in case of suspicion of plasmacytoma, for CD138. EBV detection was initially performed by EBNA-2 staining, later monoclonal antibodies against LMP were used and in situ hybridization on EBER

As confirmation of the limited antibody responses in both groups of lung transplant recipients, immunoblotting of consecutive sera was carried out in a number of representative patients.

Evaluation of the seronegative recipients with PTLD confirmed the absence of serological response in patient 1 and showed only antibodies against VCA-p18 10 months after Ltx in patient 4 (Fig. 2). Three seropositive recipients with PTLD were evaluated (Fig. 3) and five without PTLD (data not shown). In patient 17, with PTLD, serological reactivation was confirmed by immunoblot. In patient 15 and 16 there was no change in immunoblot pattern at the diagnosis of PTLD. In patient 15, the intensity of the EBNA-1 (P72) signal decreased over time, compatible with an earlier report [Riddler et al.]. The five seropositive recipients

without PTLD showed patterns similar to the patients with PTLD with a relative few EBV reactive bands mainly consisting of the VCA-specific tegument protein P18, reactivation patterns were not detected.

***Table 4**: EBV seropositive Ltx recipients with PTLD*

Patient no.	Onset of PTLD (months after Ltx)	Significant changes* in antibody titre (weeks before (-) or after (+) diagnosis of PTLD)				
		IgM		IgG		
		EA(D)	VCA	EBNA-1	EA(D)	VCA
13	65	-	-	-	-	+4 (rise)
14	39	-	-	-	-	-2 (rise)
15	12 H	-	-	-	-	-
16	6 HH	-	-	-	-	-
17	4	+0 (pos)	-	+0 (rise)	+0 (pos)	+0 (rise)
18	5	-3 (pos)	-	-	-	-
19	32	-	-	-	-	-

* conversion from negative to positive titre or at least 10EU rise in antibody titre
pos = negative becomes positive titer; rise = at least 10 EU rise in antibody titre
In patients no. 15 and 16 no serological follow-up (H diagnosis at post-mortem, HH patient died 12 days after diagnosis)
- = no serological changes.

***Figure 2**: Immunoblot analysis of EBV seronegative recipient 1 and 4. Patient 1 shows a faint EBNA-1 band shortly after Ltx due to transfused IgG. No other bands were detected for 14 weeks. In patient 4 after 56 weeks only VCA-P18 was detected.*

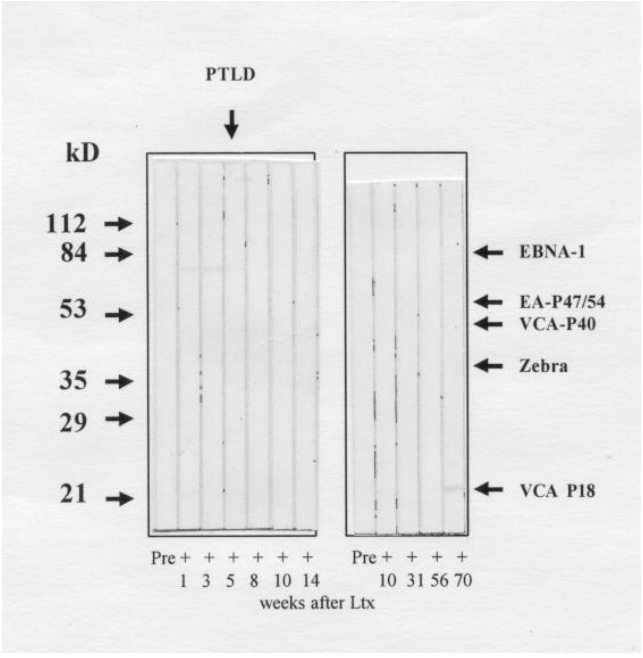
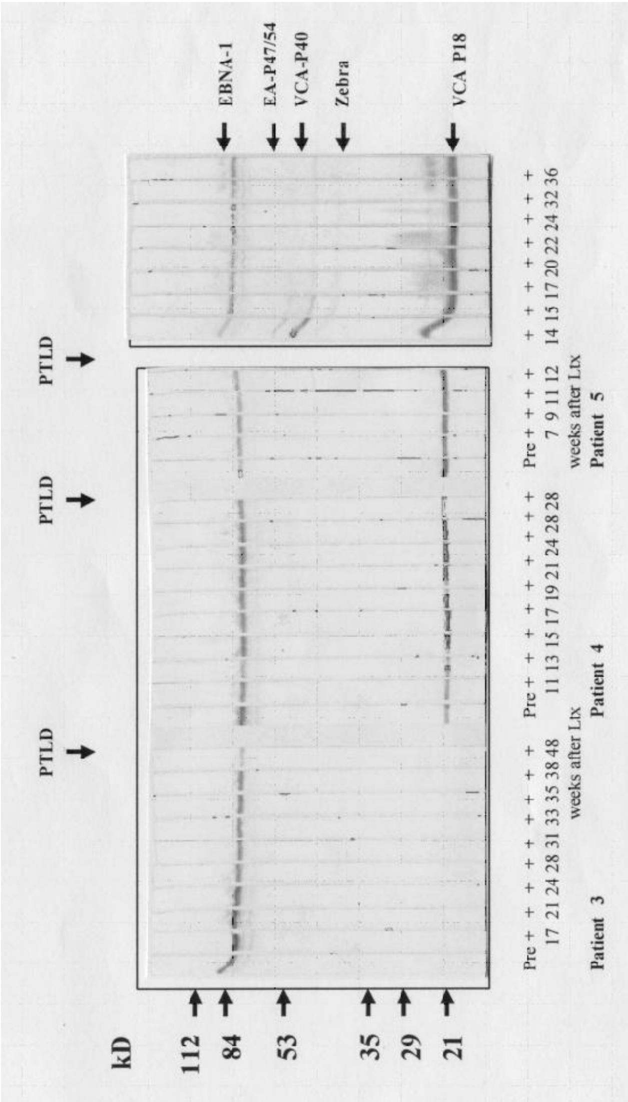


Figure 3: *Immunoblot analysis of EBV seropositive recipients 15, 16 and 17. In patient 15 only anti EBNA-1 antibodies are detected that decrease in time, in patient 16 only preexistent anti VCA-P18 and anti EBNA-1 antibodies were detected and only patient 17 showed antibodies with new specificities after PTLD*



Discussion

Our data confirm that EBV-seronegative lung transplant recipients have a significant greater risk of post-transplant lymphoproliferative disease (33% vs 7%) than do seropositive recipients. Moreover, two other trends were disease developed earlier in the former recipients (within 3 months in three of four recipients), whereas the earliest post-transplant lymphoproliferative disease in seropositive recipients were diagnosed approximately 6 months after lung transplantation. A further striking feature was the localization of the post-transplant lymphoproliferative disease: whereas the early post-transplant lymphoproliferative disease were almost all localized in the transplanted organ, the late post-transplant lymphoproliferative disease were mostly localized in the gut although disseminated post-transplant lymphoproliferative disease and other organ involvement was also seen.

The goals of our study were to evaluate the course and patterns of serological responses to EBV and their impact as a marker for early diagnosis and prognosis of post-transplant lymphoproliferative disease. In previous studies, no diagnostic role for EBV serology, using crude antigen extracts, could be shown. For this reason, we analyzed whether measurements against enriched preparations of synthetic EBV antigens would enable a sensitive detection of relevant antibodies.

IgG antibodies against the latent phase antigen EBNA-1 were not detected in any of the patients, not even when immune suppression was lowered after diagnosis of post-transplant lymphoproliferative disease. This may reflect the inability of recipient T cells to kill latently infected EBV-positive B cells effectively, which would normally lead to exposure of the nuclear EBNA-1 and subsequent induction of anti-EBNA1 antibody responses. On the other hand, appearance of anti-VCA IgM and IgG antibodies (directed mainly against the immunodominant BFRF3-encoded p18 tegument protein) would reflect active viral replication in the recipients. However, the limited response to BMRF1-encoded EA(D) and other EA and VCA proteins of the lytic phase again indicate insufficient immune responses as none of the patients with primary EBV infection did develop the full spectrum of anti-EBV antibodies characteristic of infectious mononucleosis in the immune competent host [Middeldorp and Herbrink, 1988].

Patient no. 1, who developed post-transplant lymphoproliferative disease 7 weeks after lung transplantation, achieved remission after tapering of immunosuppression and high doses of acyclovir but showed no subsequent antibodies against EBV, a clear example of the impaired serological response after quadruple immune suppression. Only after a second post-transplant

lymphoproliferative disease in the sigmoid did this patient demonstrate seroconversion. Considering cellular immune responses after the reduction of immune suppression as a possible forerunner of the initial remission this case also suggests that humoral immune responses are not an unequivocal reflection of an EBVdirected immune defense.

Taken together, the results show that in EBVseronegative recipients anti-EBV antibody responses are detectable only after the clinical diagnosis of post-transplant lymphoproliferative disease. In EBVseropositive recipients developing post-transplant lymphoproliferative disease, a rise in antibody titre was seen only in a few of the patients and even in those cases shortly before the diagnosis of post-transplant lymphoproliferative disease to be of diagnostic importance.

Consequently, this highly sensitive quantitative serology is not suitable for early diagnosis of post-transplant lymphoproliferative disease.

The antibody patterns measured by ELISA were supported by immunoblot analysis. The antibody diversity underlying the anti-EBV antibody response has proved informative for EBV-associated acute, chronic, and malignant diseases in the immune competent host [van der Giessen et al., 1990; Brink et al., 1997]. Immunoblot assays of the sera of our lung transplant recipients showed no other specificity than tested in the peptide-based ELISA, confirming the immunodominant nature of the three peptide reagents used and confirming the very restricted antibody responses in transplant recipients, similarly described by Cen et al. [1993]. Thus, antibodies against the peptide antigens used in the ELISA used in this study may be considered representative of the restricted humoral immune response against EBV in patients with severe immune suppression. In this respect lung transplant recipients are at the extreme of this spectrum.

The lack of serological response is somewhat surprising because lung transplant recipients are in general, at least partially, capable of producing antibodies to viral antigens, as detected after CMV infection. Apart from a hampered humoral immune response, this may suggest that EBV-antigens are minimally exposed, probably because they remain associated with the latently transformed cells [Wagner et al., 1995; Babcock et al., 1999]. A possible reason for a suppressed lytic cycle replication and limited presentation of EA and VCA antigens could be the acyclovir prophylaxis that is given as standard treatment to our lung transplant recipients for prevention of a-herpesvirus infection. Acyclovir has been shown to limit productive infection in vitro and reduce oral secretion of EBV in transplant recipients but has no effect on EBV genome replication and proliferation of EBV-transformed B cells [Hanto et al., 1985].

A remaining role for EBV serology could be in guiding clinical decisions, e.g., to decide when to reinstate the normal level of immune suppression after the

patient shows signs of anti-EBV immune reactivity. An extension of the current study group, however, is necessary to substantiate this suggestion.

In conclusion, anti-EBV antibody responses after lung transplantation are limited and heterogeneous, quantitative EBV serology is of limited value for the early diagnosis of post-transplant lymphoproliferative disease in both EBV-seronegative and -seropositive lung transplant recipients. At best, our results suggest that EBV serology could be used as a marker for protective immunity in EBV-seronegative recipients, but the numbers are small. To monitor EBV-induced lymphoproliferation in the transplant recipient, a direct parameter such as viral load measurement by quantitative polymerase chain reaction (PCR) [Stevens et al., 1999, 2001] is probably more relevant than an indirect parameter such as EBV serology.

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