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Lowered Immune Cell Function in Liver Recipients Recovered From Posttransplant Lymphoproliferative Disease Who Developed Graft Tolerance

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Introduction. Tolerance after treatment and recovery from posttransplant lymphoproliferative disease (PTLD) have been described but little is known about the immunology. The objective of this study is to evaluate the immunity of pediatric recipients who recovered from PTLD. **Materials and Methods.** Pediatric recipients who recovered from PTLD after liver transplant and twice the number of recipients who never had PTLD were recruited. Their immune statuses were measured by ImmuKnow (measurement of adenosine 5-triphospate level produced CD4+ T helper cells), and the results were divided into 3 groups, "low" (≤225 ng/mL), "moderate" (226 to 524 ng/mL), and "high" (≥525 ng/mL). The results of both groups were compared and analyzed. **Results.** Nine PTLD recipients and 20 non-PTLD recipients were recruited. There were no significant differences in terms of sex and age between the 2 groups. The majority of PTLD recipients (88.9%) had "low" immune status responses, and none of them had "high" responses. For non-PTLD recipients, more than half (55%) had "moderate" immune status responses. The median value of adenosine 5-triphospate levels was significantly lower in the PTLD group (119 ng/mL vs 380.5 ng/mL $P = 0.014$), and their trough immunosuppressant level was also lower (3.8 μg/L vs 7.7 μg/L; $P = 0.004$). None of the patients in either group had abnormal liver enzymes (aspartate aminotransferase/alanine aminotransferase) to suggest graft rejection. **Conclusions.** Patients who recovered from PTLD have a lower CD4 T-cell activity compared with those who have not suffered from PTLD. Under careful monitoring, their immunosuppressant levels can be kept at low levels to prevent recurrence of PTLD.

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Posttransplant lymphoproliferative disorder (PTLD) is a life-threatening complication of immunosuppression and occurred in 1% to 20% of solid organ transplant recipients.[1,2] The incidence of PTLD in adults after liver transplantation varies from about 1% to 3% in the literature.[3,4] In pediatric patients, a higher incidence of up to 6% to 20% was noted, largely attributed to the pretransplant Epstein-Barr virus seronegativity status in this group of patients.[5-8] The incidences are also believed to correlate with the dosage of immunosuppression for each type of organ transplant.[9]

Immune tolerance after treatment and recovery from PTLD in a small proportion of recipients had been described. Some recipients are even able to wean off from immunosuppressant.[10,11] The immunity of the recipients who survived PTLD however is largely unclear. An immunoassay named ImmuKnow (Cylex, Columbia, MD) has been developed and designed to measure global cell-mediated immunity in immunosuppressed populations.[12,13] This assay is approved by the US Food and Drug Administration. It is able to measure the ability of CD4+ T helper cells to respond to mitogen activation by quantifying the amount of adenosine 5-triphospate (ATP) produced by CD4+ T helper cells after stimulation. Because ATP is the basic

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TABLE 1.

Comparisons between PTLD and Non-PTLD patients in terms of demographic, trough immunosuppressant, liver enzyme and ATP levels

	PTLD (n = 9)	Non-PTLD (n = 20)	*P*
Male:female	0.8 (4:5)	0.8 (9:11)	—
Age, mo	84 (28-168)	78 (26-192)	0.05
Posttransplantation, mo	48 (10-120)	70 (8-156)	0.10
Trough immunosuppressant level, μg/L	3.8 (3-5.6)	7.7 (4.2-12)	0.004
Liver enzyme	36 (10-78)	30 (20-62)	0.07
AST, U/L	40 (15-60)	33 (15-50)	0.15
ALT, U/L			
ATP level, ng/mL	88.9% (8)	25% (5)	0.006
Low (≤225)	11.1% (1)	55% (11)	
Moderate (226-524)	0% (0)	20% (4)	
High (≥525)			

energy source of effector functions of immune cells, immune responses of immune cells can be reported by the amount of ATP (ng/mL) generated.

The objective of this study is to assess the immune status of pediatric postliver transplant recipients who recovered from PTLD and find out whether these recipients are tolerant to the liver graft. The accidental development of tolerance in these recipients who recovered from a life-threatening illness also provides the golden opportunity to understand more about tolerance in liver transplantation.

MATERIALS AND METHODS

Liver recipients from Queen Mary Hospital who recovered from PTLD (evidence by histological studies of resected specimens) and twice the number of recipients (control arm, age-matched with comparable immunosuppression, and duration from liver transplantation) who never had PTLD were recruited. Recipients with history of rejection or transplanted for autoimmune diseases were excluded. Patients with infectious mononucleosis-like cases but no histological evidence of PTLD changes were not included. During the outpatient follow-up of recruited patients and controls, 3 mL of blood was collected in the same session of routine blood tests. The blood samples were processed by the ImmuKnow (Cylex) for measurement of the CD4+ ATP levels. Clinical data of patients were retrieved from hospital computerized database.

The immune status measured by ImmuKnow will be reported as numerical value and divided into 3 levels. If the ATP level is below 225 ng/mL, the immune cell response will be considered as "low." If the ATP level is between 226 and 524 ng/mL, it will be considered as "moderate" immune cell response. A level higher than 525 ng/mL will be considered as "high" immune cell response.

Statistical analysis was performed using IBM Statistical Package for Social Science, version 20.0 (SPSS ver 20.0). Continuous variables were expressed as median (range) and compared using the Mann-Whitney *U* test. Categorical variables were compared using the χ^2 test. A *P* value less than 0.05 was considered to be statistically significant. This study has been approved by the Institutional Review Board of Queen Mary Hospital/Hong Kong West Cluster (IRB reference UW12-552).

RESULTS

Of 125 pediatric patients (age, < 18 years) who had undergone primary liver transplantation since 1993, 10 patients recovered from histologically proven PTLD, survived, and consented to participate in this study. However, a patient was excluded because she was receiving treatment for recurrent disease at the time of study. Twenty age- and sex-matched control patients were also recruited. The results are summarized in Table 1. There were no significant differences in terms of sex and age between the 2 groups. The follow-up period was longer in the non-PTLD group but this was not statistically significant. For the PTLD group, according to the World Health Organization classification, 2 patients had early diseases, 6 patients had polymorphic diseases, and 3 patients had monomorphic diseases (lymphoma-like condition). Except for 2 patients in the PTLD group, all patients were taking tacrolimus as the sole immunosuppressant, and the trough drug level at the time of this study was significantly lower in the PTLD group (3.8 μg/L vs 7.7 μg/L; $P = 0.004$). Three patients in the PTLD group were able to wean off from immunosuppression for longer than 6 months. None of the patients had abnormal liver enzyme (aspartate aminotransferase/alanine transaminase). Regarding the ATP level as measured by ImmuKnow, majority of PTLD recipients (88.9%) had "low" responses and 11.1% had "moderate" response, and none of them had "high" response. For non-PTLD recipients, 25% had "low" response, 55% had "moderate" responses, and 20% had "high" response. The median value of ATP level was significantly lower in the PTLD group (119 ng/mL, range, 36-257 ng/mL vs 380.5 ng/mL, range, 120-651 ng/mL; $P = 0.014$) (Figure 1).

DISCUSSION

The incidence of PTLD (8%) in the present series is comparable to international standard. In this study, we were able to demonstrate that a lower level of T-cell immunity is noted in these patients. Therefore, it can be interpreted that the development of tolerance and less requirement for immunosuppression in some of these patients maybe a result of lower T-cell immune response. A definite explanation for the underlying physiology will need further laboratory study to answer but this finding has major impact on the clinical

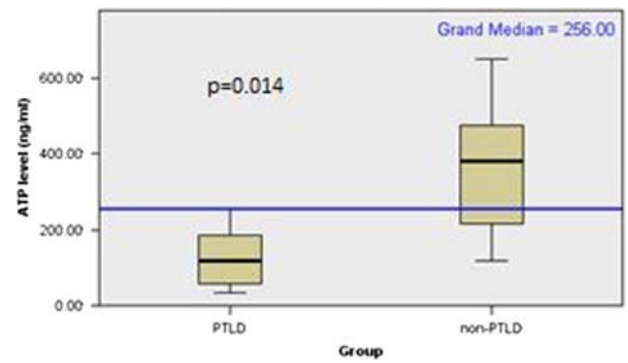

FIGURE 1. Comparison of median ATP levels between PTLD and non-PTLD recipients showing a significantly lower level in the former group.

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management of PTLD patients. Having known about the low level of T-cell activity in PTLD patients, these patients can receive a minimal dosage of immunosuppression with less fear of rejection. It can be seen that the median trough drug level was also lower in our PTLD patients, and there was no evidence of rejection (characterized by the normal liver enzyme levels). Indeed, 3 patients were able to take off immunosuppression with stable graft function, another evidence to support that a lower dosage of immunosuppressant should be considered as a safe maintenance therapy to prevent the recurrence of PTLD.

Liver transplant is an established surgical treatment for end-stage liver disease in adults as well as children. As with other organ transplantations, allogenic response after engraftment has to be suppressed by the proper use of immunosuppression to avoid graft rejection. The recognition of alloantigen by T cell is always the first step to provoke the chain of reactions leading to graft rejection. Therefore, majority of immunosuppressive drugs act on T cell. The oversuppression of T cell, however, can occasionally lead to uncontrolled proliferation of B cell which is the essential pathogenesis of PTLD.

Posttransplant lymphoproliferative disease was first recognized in the late 1960s and in majority of the cases, an association with Epstein-Barr virus infection was noted.[14-16] Other risk factors which have been reported to be associated with the its development include age of the recipient, older donors, high levels of immunosuppression, immunosuppression regimen, degree of HLA mismatching as well as the use of anti-lymphocyte therapies.[17,18] Posttransplant lymphoproliferative disease is heterogeneous group of lymphoproliferative diseases ranging from benign polyclonal B-cell proliferation to a malignant monoclonal lymphoma.[19] The disease severity has been classified by the World Health Organization in 2008 and is classified into 4 subtypes: early, polymorphic, monomorphic (B-cell/T-cell subtype), and classical Hodgkin lymphoma lesions.[20] Because the overuse of immunosuppressant is the most widely agreed reason for the development of PTLD, the accepted treatment policy for this condition is immunosuppression reduction. However, reported overall response rate to the reduction of immunosuppression varies, and at most, around 74% of patients will respond with only one third of them having sustainable response.[21-25] In addition, it may take a long time before a clinical response is evident, and therefore, adjuvant treatments and in most cases, anti-CD20 (rituximab) or chemotherapy will be needed.

Interestingly, it has been reported that some recipients who recover from PTLD will develop operational graft tolerance.[10] Tolerance is defined as the specific absence of a destructive immune response to transplanted tissue in the absence of immunosuppression and can be divided into central and peripheral. When this happens, immunosuppression can be withdrawn. Operational tolerance is defined as the absence of graft dysfunction without the use of immunosuppression. As mentioned previously, the major reason for rejection and hence barrier to tolerance is the recipient's endogenous T cell. However, little is known about why tolerance or lesser propensity for rejection (reflected by the minimal use of immunosuppression) can develop in some patients after recovery from PTLD. To answer this question, we attempted to measure the T-cell immunity in patients with a history of PTLD.

ImmuKnow is able to measure the ability of CD4+ T helper cells to respond to mitogen activation by quantifying the amount of ATP produced by CD4+ T helper cells after stimulation. Because ATP is the basic energy source of effector functions of immune cells, immune responses of immune cells can be reported by the amount of ATP (ng/mL) generated.[12,13] ImmuKnow has been assessed for the potential use in both adult and pediatric transplant patients. Some studies have reported that transplanted patients with "low" immune cell response might have a higher risk of infection, whereas patients with "high" immune cell response might have a higher risk of graft rejection although controversies exist. Nevertheless, ImmuKnow assay has been recommended for the monitoring of cell-mediated immunity after solid organ transplantation.[26-30] Because it is always difficult to titrate the level of immunosuppressant and set a balance between rejection and recurrent of PTLD, the measurement of T-cell activity can therefore be taken as an assessment of the likelihood of rejection and guide the use of immunosuppressive therapy apart from clinical and biochemical monitoring.

Limitations

This study has a few limitations. First, the sample is relatively small but this is just the normal incidence of PTLD in postliver transplant recipients. A multicenter study would be required to increase the sample size of similar studies in the future. Second, the evaluation of tolerance and rejection was limited to a 1-point analysis. Although a higher incidence of tolerance and less requirement for immunosuppression in PTLD recipients could be observed in this study, a direct causal relationship would require a longer follow-up period to be established. Third, this study only evaluated pediatric patients, and the findings may not be applicable to adult recipients.

CONCLUSIONS

In conclusion, this study has demonstrated that ImmuKnow can be used in clinical practice to evaluate the T-cell function in postliver transplant recipients. Recipients who have recovered from PTLD had a lower CD4+ ATP level and probably T-cell activity than non-PTLD recipients. It may be the underlying reason for the development of tolerance and less requirement for immunosuppression in some of the patients. However, the current study is a cross-sectional study only, and further studies will be required to confirm this relationship. An alternative explanation would be the higher sensitivity to immunosuppression in the PTLD patients. No matter what the underlying pathophysiology is, with the findings in the current study, we believe the level of immunosuppression can be kept low in PTLD recipients to prevent disease recurrence.

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