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Remarkable correlation between increased HLA-DQ antigen positive monocytes and prognosis of renal transplantation

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Abstract. Since cyclosporin A (CsA), a widely used immunosuppressive drug, strongly suppresses interleukin-2 (IL-2) secretion, it is frequently difficult to estimate T lymphocyte activation in early acute rejection. We found that, when evaluated based on HLA-DQ antigen expression, monocyte activation in the peripheral blood of renal transplantation patients was a very sharp parameter in diagosing acute rejection. All of 16 episodes of early acute rejection, which were relatively easily suppressed by steroid pulse therapy, showed a sharp increase in the proportion of HLA-DQ antigen-positive monocytes (DQ^+mono) and a quick return of DQ^+mono to previous values, along with a fall in serum creatinine levels. Since, however, HLA-DR antigen-positive T lymphocytes (DR^+T) were markedly increased over a long period in episodes of therapy-resistant and chronic rejection, their prolonged high value was regarded as a parameter indicative of poor prognosis.

Key words: Immunological monitoring – HLA-DQ positive monocytes – Monocyte – HLA-DQ antigen

The immunological monitoring of acute rejection has often been carried out on activated T lymphocytes by means of expression of DR antigen [1] and interleukin-2 (IL-2) receptor [2] or by assay of lymphokines, such as IL-2 [3]. These parameters have frequently been reported as showing false negative results [4]. Cyclosporin A (CsA), currently a leading immunosuppressive drug, strongly suppresses T lymphocyte activation by blocking IL-2 seretion [5]. It is therefore surmised that because of this, T lymphocyte activation may not be clearly observed in early acute and mild rejection.

It has been reported that, although HLA-class I, HLA-DR and DP antigens are expressed in high density on the monocyte membrane, HLA-DQ antigen is expressed only in 10–40% of the monocytes in peripheral blood [6, 7]. Its expression has been confirmed as stimulated by interferon-γ[8].

Speculating that the HLA-DQ antigen might be used as a marker for activated monocytes, we investigated the kinetics of the HLA-DQ antigen of monocytes in the peripheral blood of renal transplantation patients. Apparent correlation was found between acute rejection and incraesed HLA-DQ antigen positive monocytes.

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Patients and methods

Using 35 renal transplantation patients (32 living related donors and 3 cadaveric donors), surface markers of monocytes and lymphocytes in the peripheral blood were examined before, and 7, 14 and 28 days after transplantation. Examination was performed frequently when development of rejection was suspected. As immunosuppressive agents, cyclosporin A (CsA) and steroids were used in all patients. The same examination was also carried out in control groups of 25 healthy volunteers (11 males and 14 females, > 20 years old), 11 haemodialysis patients and 11 kidney transplant patients who had not experienced any problems for 1 year or longer after transplantation.

Surface markers investigated included CD3, CD8 and HLA-DR antigens for lymphocytes and HLA-DR and DQ antigens for monocytes, and were estimated by double-staining utilizing flowcytometry (FCM). Monoclonal antibodies used in staining were as follows:

1. Anti Leu4 + anti HLA-DR→activated T lymphocyte (DR^+T)
2. Anti Leu3a + anti Leu2a→T helper/suppressor ratio (H/S)
3. Anti LeuM3 + anti HLA-DR→$HLA\text{-}DR^+$monocyte (DR^+-mono)
4. Anti LeuM3 + anti Leu10→$HLA\text{-}DQ^+$monocyte (DQ^+mono)

Results

Values of surface markers in the control groups are shown in Table 1. DQ^+mono in the 25 healthy volunteers was 17.3% ± 7.2%, with no significant difference according to sex or age (data not shown). In contrast, DR^+mono was 91.2% ± 7.9%, which confirmed expression in the ma-

Table 1. FACS analysis of surface markers of lymphocytes and monocytes in control group. Results are expressed as mean ± SD

	Healthy persons (n = 25)	Haemodialysis patients (n = 11)	
		Before H.D.	After H.D.
DR⁺T (%)	9.2 ± 4.8	11.1 ± 5.4	7.8 ± 4.2
H/S	1.93 ± 0.68	1.98 ± 0.39	2.87 ± 0.75*
DR⁺mono (%)	91.2 ± 7.9	96.5 ± 3.0	97.6 ± 1.3
DQ⁺mono (%)	17.3 ± 7.2	28.4 ± 12.8	16.3 ± 7.3**

* $P < 0.001$; ** $P < 0.005$; as compared with before haemodialysis

jority of monocytes. In the haemodialysis patients, DQ⁺mono was 28.4% ± 12.8% before dialysis, which was higher than that in the healthy volunteers, and 16.3% ± 7.3% after dialysis. This should be taken into consideration when DQ⁺mono is measured in patients who need haemodialysis after renal transplantation.

Of the 35 renal transplantation patients who were prospectively monitored, 15 experienced no rejection. Table 2 shows data of DR⁺T and DQ⁺mono monitoring over time. DQ⁺mono showed a high value of 41.8% ± 27.0% before transplantation, which rapidly declined to 15.2% ± 11.1% and 13.0% ± 7.6% at 7 and 28 days after transplantation, respectively. In contrast, the mean value of DQ⁺mono in the 11 transplant patients who were incident free for 1 year or longer after transplantation was as low as 9.7% ± 4.5%. However, there was almost no change in DR⁺T. We, therefore, suggest that DQ⁺mono is a more accurate parameter.

There were 16 episodes of acute rejection which occurred within 3 months after transplantation and could be controlled by steroid pulse therapy, but DQ⁺mono quickly rose in all of them (Fig. 1). As serum creatinine levels were decreased with therapy, DQ⁺mono returned to previous values. In contrast, mean value of DR⁺T increased mildly when rejection occurred, and in 5 patients did not rise at all.

Interestingly, estimation of DQ⁺mono was also found to be a useful parameter for predicting the occurence of rejection (Table 3). When DQ⁺mono was 30% or more at 14 days after transplantation, acute rejection developed in 10 out of 13 patients (76.9%) within 3 months. When DQ⁺mono was less than 30% at 28 days after transplantation, acute rejection occurred in only 3 patients (16.7%). Such regular estimations of DQ⁺mono would appear to provide information useful for adjusting immunosuppressive therapy and setting a suitable date of discharge from hospital.

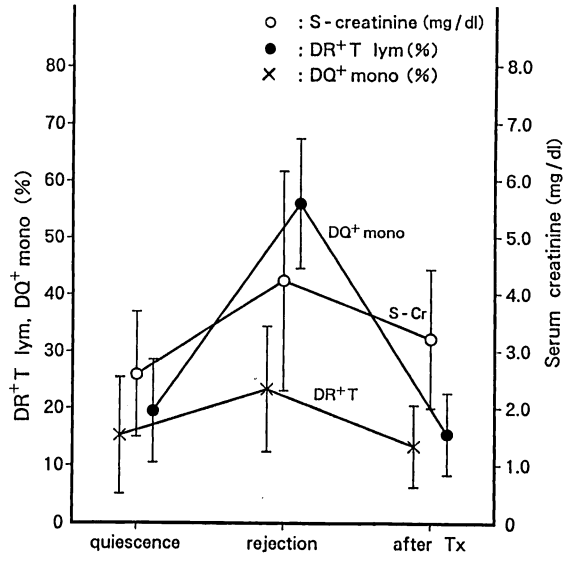

Fig. 1. Alteration of DQ⁺mono (x–x) and DR⁺T (●–●) in acute rejection of renal allograft recipients (n = 16)

Ten episodes of intractable rejection resistant to steroid pulse therapy were experienced. Of note was a prolonged increase in DR⁺T, unresponsive to pulse therapy (Table 4). In contrast, DQ⁺mono decreased in response to therapy temporarily, despite lack of improvement in renal function. Intractable persistent rejection apparently showed a prolonged increase in activated T lymphocytes, which was presumably a sign of poor prognosis.

Deteriorated renal function due to CsA nephrotoxicity was confirmed by biopsy in 5 patients, their DQ⁺mono values remaining low (Table 5). DQ⁺mono did not increase at the time of viral infection in 2 patients. In 2 patients diagnosed by biopsy as having chronic rejection, DQ⁺mono showed low values, but DR⁺T was as high as 50% or more. Therefore, DQ⁺mono increase is assumed to be a phenomenon specific to acute rejection.

Discussion

Monocytes in the peripheral blood have been classified into 2 subsets depending on the presence of DQ⁺mono [6, 7]. It has been confirmed that DQ⁻mono are transformed into DQ⁺mono by treatment with interferon-γ [8, 9]. Therefore, it is assumed that they are derived from the same origin, and that their DQ antigen expression reflects

Table 2. Serial monitoring of DR⁺T and DQ⁺mono in renal allograft recipients (n = 15) who experienced no rejection. Results are expressed as mean ± SD. Pre-Tx analysis were done before the use of immunosuppressive drugs

	Recipients					Normal healthy group (n = 25)
	Stable graft function (n = 15)				Long-term surviving graft (n = 11)	
	pre Tx	post Tx-7 days	14 days	28 days		
S-Cr (mg/dl)	10.9 ± 3.0	2.1 ± 1.6	1.6 ± 0.6	1.4 ± 0.5	1.4 ± 0.2	
DR⁺T lym (%)	8.7 ± 4.5	8.2 ± 6.6	8.3 ± 5.8	12.1 ± 8.3	11.5 ± 6.3	9.2 ± 4.8
DQ⁺mono (%)	41.8 ± 27.0	15.2 ± 11.1	15.0 ± 11.1	13.0 ± 7.6	9.7 ± 4.5	17.3 ± 7.2

Table 3. Correlation between incidence of acute rejection occurring within 3 months after renal transplantation and HLA-DQ$^+$ mono in recipients ($n = 35$) on 14th and 28th post-operative days

HLA-DQ$^+$ monocytes	Incidence of acute rejection	
On 14th day after Tx		
≦30%	6/22 (27.3%)	$\chi^2 = 8.13$ ($P < 0.01$)
>30%	10/13 (76.9%)	
On 28th day after Tx		
≦30%	3/18 (16.7%)	$\chi^2 = 12.58$ ($P < 0.01$)
>30%	13/17 (76.5%)	

Table 4. Alteration of DR$^+$T and DQ$^+$mono in acute rejection of renal allograft recipients ($n = 10$) in whom anti-rejection therapy was not effective. Results are expressed as mean ± SD

	Quiescence	After anti-rejection Tx
S-Cr (mg/dl)	3.2 ± 1.0	4.6 ± 1.4
DR$^+$T lym (%)	24.7 ± 10.1	47.5 ± 7.6
DQ$^+$mono (%)	30.1 ± 14.4	13.6 ± 9.4

Table 5. Flowcytometric data of DR$^+$T and DQ$^+$mono in renal allograft recipients with CsA nephrotoxicity, viral infection and chronic rejection

		S-CR (mg/dl)	DR$^+$T (%)	DQ$^+$mono (%)
CyA nephrotoxicity	①	2.7	11.0	21.1
	②	2.6	16.3	5.1
	③	3.7	18.5	19.3
	④	3.9	15.2	7.3
	⑤	3.5	8.1	26.6
Viral infection	①	1.1	2.8	2.7
	②	1.4	8.6	8.8
Chronic rejection	①	4.0	67.9	24.6
	②	7.3	54.2	7.7

cellular maturation or activation. The strong correlation between acute rejection and DQ antigen expression, as shown by our data, corroborated the finding that the DQ antigen is a surface marker of activated monocytes.

It has been reported that DQ$^+$mono, when compared with DQ$^-$mono, possess very strong antigen presenting ability and strong stimulation activity in mixed lymphocyte cultures [10]. It is not known how DQ$^+$mono increase in rejection functions. Possibly, however, they not only increase antigen presenting ability but also the DQ$^+$mono may act as the direct effector cells on target organs.

As shown by our data, DQ$^+$mono, when compared with DR$^+$T, also increased by responding sharply to mild rejection. This indicated interferon-γ secretion from helper T lymphocytes in the early stage of rejection and in the state of insufficient immunosuppression. We suggest that monocytes might respond sharply to interferon-γ; therefore, it would appear that the indirect estimation of activated monocyte increase would be clinically more useful than the direct measurement of interferon-γ.

DR$^+$T may be a paramater of advanced rejection and not a very useful paramater for early detection. A prolonged increase in DR$^+$T was found in frequent and chronic rejection, which did not tend to decrease with pulse therapy. The authors therefore regard it as a paramater of poor prognosis.

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