

## Immunosuppression, eotaxin and the diagnostic changes in eosinophils that precede early acute heart allograft rejection

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### Abstract

Peripheral blood eosinophil counts (EOS) are undetectable in 40% blood samples sent for routine haematology at Papworth Hospital during the first 3 months after heart transplantation (HTx). Increases in EOS usually precede the development of allograft rejection by a median of 4 days. We compared the effects of cyclosporin (dose and total blood concentration), prednisolone (dose and both total and unbound plasma concentrations) and azathioprine, as well as plasma concentrations of the CCR-3 chemokines, eotaxin and RANTES, on changes in EOS in 47 consecutive HTx recipients, with a median follow-up of 90 (IQR 85–95) days. Multivariate analysis confirmed the independent association between both prednisolone dose ( $P < 0.0001$ ) and eotaxin ( $P < 0.0001$ ) and changes in EOS. The plasma eotaxin concentration was, in turn, most closely associated with the cyclosporin dose ( $P < 0.001$ ) and plasma prednisolone concentration ( $P = 0.022$ ). The blood cyclosporin concentration ( $P = 0.028$ ), EOS ( $P = 0.012$ ) and prednisolone dose ( $P = 0.015$ ) were all independently associated with the risk of treated acute rejection. When prednisolone pharmacokinetic parameters were substituted for the prednisolone dose in this multivariate model, only the pharmacokinetic parameter retained a significant association with the risk of rejection. Changes in EOS preceding cardiac allograft rejection are directly associated with plasma eotaxin concentrations and indirectly with prednisolone dosage. Cyclosporin may also indirectly influence these changes by inhibiting eotaxin production. EOS, prednisolone dose and blood cyclosporin concentrations were independently associated with the risk of acute rejection. The total and unbound fractions of prednisolone in plasma appear to be even more closely related to rejection but are difficult to measure. Monitoring EOS, as a surrogate measure of prednisolone immunosuppression, may be more cost-effective for controlling rejection than conventional cyclosporin monitoring in the first 6 weeks after HTx.

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### 1. Introduction

The common association between a peripheral blood eosinophilia and the clinical diagnosis, 1–4 days later, of acute cellular rejection of solid organ allografts was first recognised in the kidney transplant population [1].

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This report has been followed by very similar longitudinal observations in liver [2], heart and lung transplant populations [3]. It has also become recognised that eosinophils are a prominent component of the inflammatory infiltrates seen in acute allograft rejection. Indeed, liver allograft infiltration by eosinophils has been referred to as a cardinal diagnostic feature of clinical rejection as the degree of infiltration was found

to correlate with the severity of rejection [4]. A concomitant increase in serum eosinophil cationic protein concentration has also been found in association with the eosinophilia preceding liver allograft rejection but, unlike the graft infiltrate, neither laboratory test was found to correlate with the histological grade of rejection [5].

The diagnostic and pathogenic significance of the increases in peripheral blood eosinophils associated with allograft rejection are currently being most actively addressed in the context of both human and experimental heart transplantation. Although the changes in peripheral blood eosinophil counts (EOS) preceding the clinical diagnosis of human heart transplant (HTx) rejection are very modest when compared with the frank eosinophilia found in the other transplant groups, these changes uniquely correlate with the histopathological grade of rejection [3]. Crucially, it was also confirmed that these diagnostic changes in eosinophils are not found in association with clinically significant episodes of infection following heart transplantation. The diagnostic potential of this test is particularly great in this population as there are no other laboratory markers that can be used routinely to prompt the early treatment of clinical rejection (International Society of Heart and Lung Transplantation Grade  $\geq 3A$ ). In a randomised clinical trial of EOS monitoring, we established that this simple and cheap test can be used to prompt early augmentation of low-dose intravenous steroid therapy and so halve the rate of treated HTx rejection within the first 6 post-operative weeks [6].

Observations in a murine model of HTx rejection suggest that eosinophils play a direct role as mediators of allograft rejection under the regulation of CD8(+) lymphocytes and interferon-gamma [7]. An independent group has subsequently found that different CD8+ T cell subsets, with distinct chemokine receptors, can elicit different histopathological forms of murine HTx rejection and the Tc2 subset that produces high levels of IL-4 and IL-5 promotes recruitment of secondary effector cells—particularly eosinophils [8].

If eosinophils are secondary effector cells in human heart allograft rejection, a fuller understanding of the regulation of their recruitment may provide insight into improved methods for controlling their pathogenic function. We have already established that the administered doses of prednisolone (PRL) correlate weakly with concurrently measured EOS during the first 3 post-operative months although EOS was more closely associated with the clinical end-point - treated rejection [3]. This was the basis for using EOS as an intermediate therapeutic end-point for PRL dosage (PRL<sub>d</sub>) adjustment in our clinical trial [6] but also provides the rationale for using EOS to investigate the factors regulating eosinophil recruitment in the context of HTx rejection. Wide inter-individual variability in PRL phar-

macokinetics has already been highlighted as one factor that could explain variable responsiveness to corticosteroids following both intravenous therapy in kidney [9] and oral therapy in lung [10] transplant recipients and monitoring PRL concentrations was advocated to help guide dosage adjustment.

The very distinctive magnitude of the changes in EOS associated with rejection of different solid organ allografts, particularly when comparing thoracic and abdominal organs, could provide a clue to the mechanisms controlling this eosinophil mobilisation. Although PRL<sub>d</sub> regimens do differ between these transplant groups, it is probable that the other immunosuppressive drugs used in conjunction with steroids exert at least some influence on eosinophil activation. For example, cyclosporin (CYA) has been shown to have an inhibitory effect on eosinophil-associated cytokines and chemokines [11,12]. The target pre-dose total whole blood CYA concentration (CYA<sub>t</sub>) measurements in different transplant populations are particularly variable and tend to be much greater in recipients of thoracic allografts [13].

We have reviewed longitudinal clinical and laboratory data (including blood CYA and plasma PRL concentrations) collected prospectively during the first 3 post-operative months from 47 consecutive heart transplant recipients [6]. In addition, we have measured plasma concentrations of the CCR-3 chemokines, RANTES (regulated upon activation, normal T cell expressed and secreted) and eotaxin, both of which are known to influence eosinophil function. RANTES has already been implicated in the pathogenesis of acute cardiac cellular rejection [14,15]. Its chemotactic activity is not specific for eosinophils, its receptors exhibiting considerable promiscuity, and it can also attract T-cells and macrophages—acting as a haptotactic mediator at the endothelium to recruit more inflammatory cells into the graft [16]. Eotaxin also has the capacity to selectively prime eosinophils for chemotaxis, to direct their migration/chemotaxis, and to activate inflammatory activity in the cells attracted. However, in contrast to RANTES, eotaxin activity is directed exclusively through the CCR-3 receptor and it influences only those cell types that express this receptor—namely, eosinophils, basophils, mast cells and a subpopulation of Th2-type T-lymphocytes [17]. Eotaxin can induce both the local recruitment of eosinophils from the microcirculation and also rapid mobilisation of bone marrow eosinophils, in synergy with interleukin-5. The relationship between these data and both EOS and the clinical diagnosis of acute allograft rejection has been explored by regression analysis.

## 2. Objective

Despite the potential clinical merits of pharmacodynamic tests in monitoring immunosuppression in trans-

plant recipients and numerous attempts to develop such techniques, none has received widespread application. This has been attributed to the time-consuming and costly nature of most pharmacodynamic monitoring methods as well as a lack of evidence from prospective studies that this supplementary monitoring improves clinical outcome [18]. The simple and cost-effective method of monitoring EOS as a guide to corticosteroid therapy is proving to be an exception to this gloomy picture. Adjusting steroid dosage according to changes in EOS halves the rate of treated allograft rejection within the first 6 weeks after heart transplantation [6]. However, we do not understand either the immunological mechanism underlying this pharmacodynamic relationship or the impact of the other immunosuppressive drugs used in combination regimens on this mechanism. The aim of this study was to unravel the complex relationship between combination immunosuppressive drug therapy and the pharmacodynamic and prognostic changes in eosinophils associated with allograft rejection. The role of CCR3 chemokines, including eotaxin with its exquisite specificity for eosinophils, in this relationship was also explored.

### 3. Materials and methods

#### 3.1. Patients

The first 47 consecutive HTx recipients recruited to our trial of EOS monitoring [6], with a median follow-up of 90 (IQR 85–95) days, were studied. EDTA anticoagulated blood samples for the measurement of whole blood CYA, plasma corticosteroids and chemokines were collected daily throughout the in-patient period and then at every out-patients visit until the end of the third post-operative month. Platelet-free plasma was prepared by microcentrifugation at  $10\,000\times g$  for 10 min as soon as possible after venepuncture and the plasma stored at  $-20\text{ }^{\circ}\text{C}$  until assay.

Of the 47 HTx recipients included in our review, 36 (78%) were male and they had a mean (S.D.) age of 51 (9) years. The median (inter-quartile range) length of follow-up was 90 (85, 94) days. The cumulative follow-up for all 47 patients was 4160 days. We excluded the 429 days coinciding with the first 7 days post transplantation, when the measurements were likely to be influenced by intra- or peri-operative blood transfusions. A total of 3731 days remained with EOS available on 1161 days for statistical analysis.

##### 3.1.1. Rejection

The definition of acute allograft rejection was based upon the requirement for treatment with high dose intravenous methyl-prednisolone. It was usually diagnosed from the histological examination of protocol endomyocardial biopsies and the severity of rejection

was graded semi-quantitatively according to published histopathological criteria (International Society for Heart and Lung Transplantation grading system [19]). Only patients with biopsies showing grade  $\geq 3$  rejection were treated. When a biopsy rejection grade could not be ascribed, either because of inadequate sampling or atypical features such as superimposed peritransplant injury, treatment was given if there was clinical concern that there might be significant rejection present.

#### 3.2. Corticosteroid assays

The pre-dose total plasma PRL ( $\text{PRL}_t$ ) concentration was measured by a validated HPLC method [20]. The unbound fraction of prednisolone ( $\text{PRL}_{fu}$ ) was measured by means of an ultra-filtration technique. Firstly, this involved the purification of [2, 4, 6, 7- $^3\text{H}$ ]-labelled PRL (Amersham, Buckinghamshire, UK) by preparative HPLC in order to eliminate small amounts of radiochemical contaminants.

##### 3.2.1. Purification of [2,4,6,7- $^3\text{H}$ ]-prednisolone

The chromatographic system consisted of a Supelcosil LC-18-DB,  $150\times 4.6$  mm, 5- $\mu\text{m}$  particle size, reverse-phase column maintained at ambient temperature. The mobile phase comprised a mixture of methanol/isopropanol/water (5:20:75%) containing 0.1% v/v trifluoroacetic acid set at a flow rate of 1.2 ml/min. The detector wavelength was set at 254 nm and the retention time of PRL was found to be 10 min. A 50  $\mu\text{l}$  aliquot of [2,4,6,7- $^3\text{H}$ ]-PRL in mobile phase, containing 50  $\mu\text{Ci}$ /ml of [ $^3\text{H}$ ]-PRL with a specific activity of 40 Ci/mmol was injected into the HPLC system. The eluate was collected when the PRL peak was indicated by the UV detector and re-injected into the HPLC system followed by collection of eluate in fractions taken at 30 s intervals. It was found that the purity of the [ $^3\text{H}$ ]-PRL prepared in this way was greater than 99.9% as compared to the purity of the commercial product supplied by Amersham of 92.1%.

##### 3.2.2. Ultra-filtration method

A twenty microliter aliquot of purified [ $^3\text{H}$ ]-PRL in mobile phase was added to 1 ml plasma to yield a radioisotopic activity of 10 000 dpm per 100  $\mu\text{l}$  of plasma sample. This mixture was incubated at  $37\text{ }^{\circ}\text{C}$  for 30 min and then 900  $\mu\text{l}$  was loaded into Amicon Centrifree<sup>®</sup> ultra-filtration devices assembled with regenerated cellulose membranes with a molecular weight cut-off of 30 kD (Millipore, Watford, UK). The ultra-filtration devices were then centrifuged at  $37\text{ }^{\circ}\text{C}$  in a KR 4.22 centrifuge (Jouan, Saint-Herblain Cedex, France) at  $2000\times g$  for 45 min. The radioisotopic activity in both 100  $\mu\text{l}$  of the original plasma sample/radio-labelled drug mixture (total count) and 100  $\mu\text{l}$  ultrafiltrate was measured in a liquid scintillation counter (Tri-Carb model 19 000CA,

Table 1

Data availability for regression analyses; including key to covariate abbreviations and incremental changes assigned to each covariate for assessment of comparative effects in regression models

Covariates influencing: (covariate key; significant incremental change):	EOS (patients/days)	Rejection (rejection/clear periods)
Total plasma PRL concentration (PRL <sub>t</sub> ; 30 µg/l)	44/626	29/212
Unbound PRL fraction (PRL <sub>fu</sub> ; 3%)	43/574	27/192
Unbound PRL concentration (PRL <sub>cu</sub> ; 1.5 µg/l)	43/574	27/192
PRL Dose (PRL <sub>d</sub> ; 0.1 mg/kg)	47/4021	47/476
Cyclosporin concentration (CYA <sub>t</sub> ; 100 µg/l)	47/756	43/242
Cyclosporin dose (CYA <sub>d</sub> ; 0.5 mg/kg EOS, 1 mg/kg rejection)	47/4013	47/476
Azathioprine dose (AZA <sub>d</sub> ; 0.4 mg/kg)	47/4030	45/468
Eotaxin concentration (50 µg/l)	44/982	13/101
RANTES concentration (4500 µg/l)	38/1109	32/222
EOS ( $0.01 \times 10^9/l$ )	NA	46/304

Packard Instrument Company, Downers Heights, IL, USA). PRL<sub>fu</sub> was calculated by dividing the radioactivity count in the ultra-filtrate by the total count. The unbound concentration of prednisolone (PRL<sub>cu</sub>) was then derived by multiplying the PRL<sub>t</sub> by PRL<sub>fu</sub>.

### 3.3. CYA<sub>t</sub> immunoassay

CYA<sub>t</sub> was measured by a commercially available immunoassay (EMIT, Dade-Behring, Milton Keynes, UK), selective for the parent drug.

### 3.4. Plasma eotaxin and RANTES immunoassay

Both chemokines were measured in plasma by commercially available immunometric assays (Quankine Colourimetric Sandwich ELISA; R & D Systems, Abingdon, UK).

### 3.5. Statistical analysis

Statistical analysis was carried out by an independent biostatistician. Only days when EOS was recorded within 5 days before rejection could be included in the analysis and there was also some variability in the concurrence of covariates and EOS data. Data from the first post-operative week were also excluded, as these were likely to be influenced by peri-operative blood transfusions. A summary of available covariate information is shown in Table 1. Univariate and multivariate regression analyses, allowing for within- and between-

patient correlations (Generalised Estimating Equations (GEE)) [21] were used to identify covariates having a significant association with EOS, eotaxin and rejection. The Wald test was used to assess statistical significance in all models.

#### 3.5.1. Covariates influencing EOS

As the EOS data were significantly skewed an appropriate transformation could not be found, and so the data were dichotomised into undetectable ( $< 0.01 \times 10^9/l$ ) and detectable ( $\geq 0.01 \times 10^9/l$ ). Logistic regression (GEE approach) was then used to assess the effects of covariates on EOS. We considered the effects of CYA<sub>d</sub>, CYA<sub>t</sub>, PRL<sub>d</sub>, PRL<sub>t</sub>, PRL<sub>fu</sub>, PRL<sub>cu</sub> and AZA dose (AZA<sub>d</sub>), as well as plasma concentrations of eotaxin and RANTES on changes in EOS after HTx. The relative size of the effects of these covariates on EOS (and rejection) was assessed by comparing their odds ratios (OR) for an incremental increase in each covariate that approximated the difference between the lower quartile and median values for that covariate (Table 1).

#### 3.5.2. Covariates influencing eotaxin

Eotaxin values were log transformed before regression analysis. Results are reported as a percentage change (+ or -) along with 95% confidence interval (CI). We assessed the association of CYA<sub>d</sub>, CYA<sub>t</sub>, PRL<sub>d</sub>, PRL<sub>t</sub>, PRL<sub>cu</sub> and AZA<sub>d</sub> with eotaxin.

#### 3.5.3. Covariates influencing rejection

Data were divided into rejection (the day of rejection was taken to be the end of a 6 day rejection period)

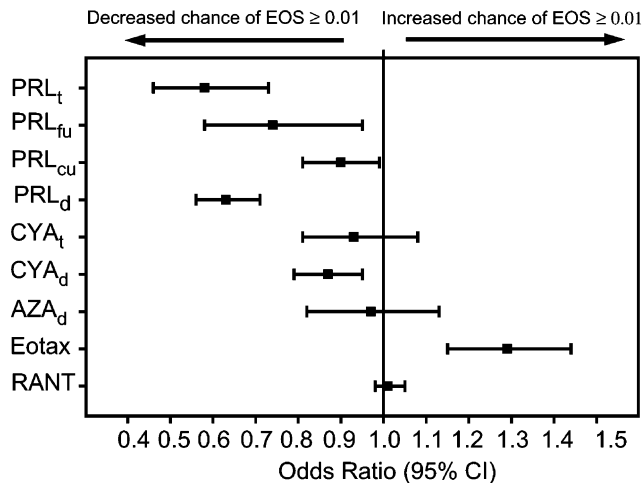


Fig. 1. Summary of univariate regression analyses between selected covariates and EOS during the first 3 post-operative months in 47 HTx recipients. The upper 95% CI of the OR for covariates that were associated with a decreased chance of  $\text{EOS} \geq 0.01 \times 10^9/l$  (e.g. PRL<sub>t</sub>) is less than 1.0. The lower 95% CI of the OR for covariates associated with an increase in the chance of  $\text{EOS} \geq 0.01 \times 10^9/l$  (only eotaxin) is greater than 1.0.

and clear (rejection-free) 6 day periods. The 5 days after a rejection episode and 3 days after any intravenous steroid therapy were excluded from analysis as 'wash-out' periods. The final data set included 47 rejection periods and 476 clear periods. Logistic regression was used for this analysis, considering the same covariates as those included in the analyses above. Results are reported as OR (95% CI).

In all three analyses multivariate models included adjustment for EOS monitoring in the reporting group during the randomised trial [11].

## 4. Results

### 4.1. Covariates influencing EOS

Univariate analysis did not reveal any significant relationships between, CYA<sub>t</sub>, AZA<sub>d</sub> or plasma RANTES concentration and EOS and these covariates were excluded from further analysis. The covariates that were most closely related to changes in EOS included PRL<sub>d</sub> (inverse relationship) and the eotaxin (direct relationship) concentration (Fig. 1; both  $P < 0.0001$ ). Their independent influences on EOS were confirmed by multivariate analysis. Because PRL<sub>d</sub> was correlated with the PRL pharmacokinetic parameters (PRL<sub>t</sub>, PRL<sub>fu</sub>, PRL<sub>cu</sub>), they were modelled separately. Also, the technical difficulties involved in measuring these parameters in the routine laboratory makes them less clinically relevant. The results are shown in Table 2. When PRL pharmacokinetics parameters, PRL<sub>t</sub>, PRL<sub>fu</sub> or PRL<sub>cu</sub> were substituted for PRL<sub>d</sub>, the size of the effect of eotaxin in the model was reduced (from odds ratio 1.26 to 1.17; and  $P = 0.025$  to 0.05).

### 4.2. Covariates influencing eotaxin

In view of the relationship between eotaxin concentrations and EOS, an additional analysis was done to assess which covariates might be influencing concentrations of this chemokine. Only two covariates, CYA<sub>d</sub> and PRL<sub>t</sub>, were significantly (both inversely) associated with changes in eotaxin by univariate analysis and their independent association was confirmed in a multivariate model (see Table 2).

### 4.3. Covariates influencing rejection

The results of univariate logistic regression analysis to identify covariates associated with risk of rejection

Table 2  
Summary of multivariate regression analyses to assess covariates associated with EOS, eotaxin and acute rejection

Factor influenced by covariates	Covariate (increment)	Effect (95% CI)*	P-value
EOS	Eotaxin (per 50 $\mu\text{g}/l$ )	1.26 (1.13, 1.42)	<0.0001
	PRL <sub>d</sub> (per 0.1 mg/kg)	0.65 (0.56, 0.76)	<0.0001
Eotaxin	CYA <sub>d</sub> (per 0.1 mg/kg)	-7% (-10, -3%)	<0.001
	PRL <sub>t</sub> (per 30 $\mu\text{g}/l$ )	-5% (-9, -2%)	0.022
Rejection	CYA <sub>t</sub> (per 100 $\mu\text{g}/l$ )	0.77 (0.61, 0.97)	0.028
	PRL <sub>d</sub> (per 0.1 mg/kg)	0.72 (0.55, 0.94)	0.015
	EOS (per $0.01 \times 10^9/l$ )	1.07 (1.02, 1.13)	0.012

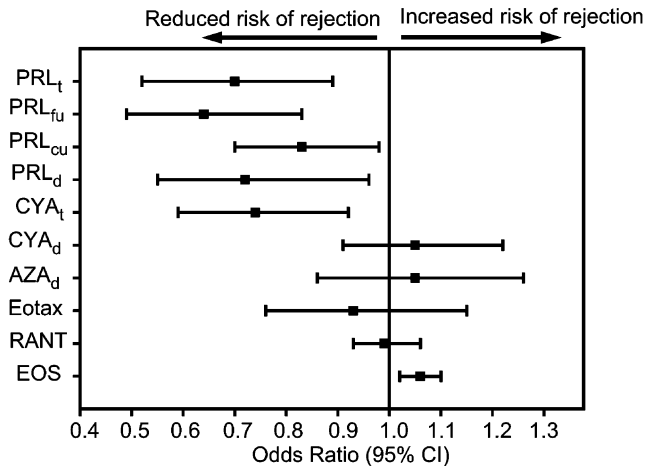


Fig. 2. Summary of univariate regression analyses between selected covariates and treated acute rejection during the first 3 post-operative months in 47 HTx recipients. The upper 95% CI of the OR for covariates associated with a significant decrease in the risk of rejection (e.g. PRL<sub>fu</sub>) is less than 1.0 (vertical broken line). The lower 95% CI of the OR for covariates associated with a significant increase in the risk of rejection (only EOS) is greater than 1.0.

are shown in Fig. 2. All PRL pharmacokinetic parameters were associated with the risk of rejection but PRL<sub>fu</sub> had the largest effect. Multivariate analyses, excluding prednisolone pharmacokinetics parameters (PRL<sub>t</sub>, PRL<sub>fu</sub>, PRL<sub>cu</sub>), showed that the covariates that were independently associated with the risk of rejection in multivariate analysis were PRL<sub>d</sub>, CYA<sub>t</sub> (both inverse relationships) and EOS (direct relationship) (see Table 2). However, when any of the PRL pharmacokinetics parameters were substituted for PRL<sub>d</sub> in the model, only the individual pharmacokinetics parameter retained an association with rejection (PRL<sub>t</sub>,  $P=0.046$ ; PRL<sub>fu</sub>,  $P=0.003$ ; PRL<sub>cu</sub>,  $P=0.057$ ). The size of the effect of PRL<sub>fu</sub> may be related to the non-linear binding of the drug over the dose range that is used therapeutically to control rejection (Fig. 3) but it is not clear why the effect of the pharmacologically active component, PRL<sub>cu</sub>, was not greater than that observed in this respect.

## 5. Discussion

Acute allograft rejection remains the most common complication after heart transplantation and is a major cause of morbidity and mortality. Despite a vast literature on the mechanism of this alloimmune response, few lines of research have led to significant improvements in the non-invasive diagnosis of rejection. During the early post-operative period, when the risk of rejection is greatest, markers of immune activation have generally failed to distinguish between rejection and infection and markers of graft damage, even the troponins, have lacked diagnostic sensitivity at this time [22].

We have already established that the simple EOS may have both adequate sensitivity and specificity to offer clinical utility in the differential diagnosis of early acute rejection and infection [3]. Provided it is measured with suitably sensitive methods, EOS is a highly cost-effective guide to steroid therapy [6] and its high negative predictive value for both heart and lung allograft rejection may in the future be exploited to reduce the requirements for invasive endomyocardial and transbronchial biopsy procedures.

The role of the eosinophil as a secondary effector cell in the alloimmune response is being carefully unravelled in experimental models [7,8,23] but little is known about this process in man. Our clinical diagnostic studies have now been extended to explore the immuno-pharmacological mechanisms that control the changes in EOS preceding heart allograft rejection in man. The results of these two contrasting approaches already share common features—particularly the critical role of CCR3 chemokines in the regulation of eosinophil activation. We have found that changes in EOS preceding heart allograft rejection are directly related to plasma eotaxin concentrations and inversely related to PRL dosage. To

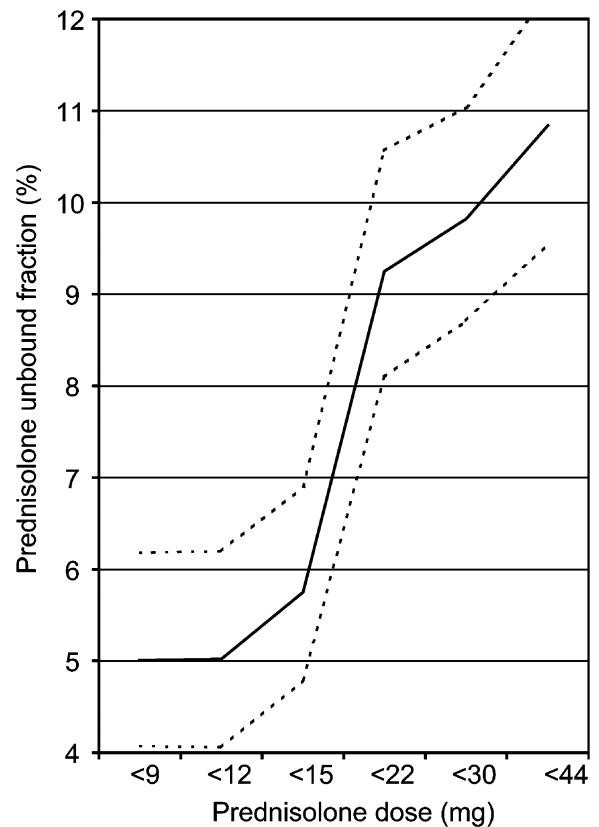


Fig. 3. Graph illustrating the increase in PRL<sub>fu</sub> (mean  $\pm$  95% CI) with increase in the split oral PRL<sub>d</sub> in 47 HTx recipients. Analysis of variance showed that the first significant increase in PRL<sub>fu</sub> occurred at PRL<sub>d</sub> greater than 15 mg ( $P<0.001$ ; adjusted for body weight, repeated measures within patients and for multiple statistical tests).

our knowledge, this is the first evidence of a direct relationship between plasma concentrations of a CCR3 chemokine and pathological changes in blood leukocyte numbers in man. The reduction in the size of the effect of eotaxin on EOS when the PRL pharmacokinetics parameters,  $PRL_t$ ,  $PRL_{fu}$  or  $PRL_{cu}$ , were substituted for  $PRL_d$  suggested that these parameters may be even more closely associated with changes in EOS than eotaxin—indicating that other steroid-related, eotaxin-independent pathways are involved in changes in EOS after HTx. The existence of such a pathway has already been suggested from experimental studies of eosinophilic pulmonary inflammation [24].

Eosinophils constitutively express CCR3 and it is this receptor that is mainly responsible for their migration into tissues. Eotaxin is the specific ligand for CCR3 and, as such, is the most potent chemoattractant for eosinophils. We, therefore, carried out further regression analyses to establish which immunosuppressive drugs influenced changes in the serum concentration of this chemokine. Although EOS is not directly influenced by CYA, we found that the  $CYA_d$  is inversely associated with eotaxin concentrations.  $PRL_t$  was also inversely and independently associated with the eotaxin concentration. Both CYA and PRL may, therefore, indirectly influence EOS through the inhibition of eotaxin production. This is consistent with literature on experimental models of eotaxin expression by human lung epithelial cells, showing the inhibitory effects of glucocorticoids and cyclosporin on both eotaxin mRNA and protein production [25,26].

$EOS$ ,  $PRL_d$  and  $CYA_t$  (but not  $AZA_d$ ) were all independently associated with the risk of acute HTx rejection within the 6 days prior to and including the day of rejection during the first 3 post-operative months. Given the low cost of EOS measurement, this indicates that EOS monitoring is even more cost-effective than conventional  $CYA_t$  monitoring in terms of the control of rejection in the early post-operative period. Again, when PRL pharmacokinetic parameters were substituted for  $PRL_d$ , only the  $PRL_t$ ,  $PRL_{fu}$  and  $PRL_{cu}$  retained an independent association with the risk of acute HTx rejection. Oddly,  $PRL_{fu}$  rather than  $PRL_{cu}$  had the largest effect on the risk of rejection but we do not have an explanation for this yet.

The model of acute eosinophilic heart allograft rejection proposed by Michel Goldman and colleagues [27] can now be further annotated to include the inhibitory effects of CYA and PRL in the modulation of those cytokine/chemokine driven events that may culminate in eosinophil degranulation within the allograft (Fig. 4). These proposed effects are again consistent with observations made in studies of the allergic immune response—both glucocorticoids and cyclosporin influencing T-cell function and eosinophil activation via inhibition of Th2 cytokine (IL-4, IL-5, IL-13) gene

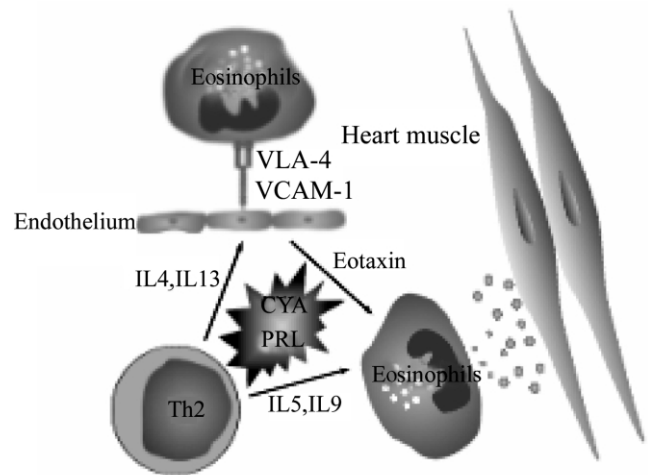


Fig. 4. Schematic model of acute eosinophilic heart allograft rejection (after Goldman et al. [27]) showing interaction of immunosuppressive drugs, CYA and PRL.

and/or protein expression [11,28–30]. The independent effects of CYA and PRL on acute eosinophilic rejection may also explain our observation that EOS measurements tend to be much lower in thoracic organ transplant recipients than recipients of abdominal allografts since they are generally maintained on higher  $CYA_t$ . However, there is one troublesome alternative explanation for this observation. We have found that EOS measurements made on different haematology analysers can give higher results than the Bayer H3 analyser used for these studies. This is probably due to contamination by neutrophils in the flow cytometric quantitation of EOS. Since blood neutrophil counts and EOS tend to change in a reciprocal manner during steroid treatment, such contamination leads to a complete loss of any clinical correlation between EOS and heart allograft rejection (data not shown). This may also provide an explanation for the failure of other transplant centres to recognise the prognostic significance of changes in EOS if they do not use specific methods for measuring the very low EOS that are detectable during steroid therapy.

The cost of EOS monitoring during the first 6 weeks after heart transplantation amounts to less than \$50 per patient [6]—about one-tenth of the cost of CYA monitoring during the same period. The results of this study confirm that therapeutic monitoring of PRL by EOS measurement may be more cost-effective than conventional monitoring of CYA treatment in the control of acute allograft rejection. The results also provide insight into the mechanism of EOS activation preceding heart allograft rejection and the potential of novel drug therapies, such as selective CCR3 antagonists.

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