RESPIRATORY MEDICINE (1999) **93**, 119–122

No relation of tuberculin reactivity with quantitative analysis of peripheral blood lymphocyte subsets in haemodialysis patients

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Anergic response to tuberculin (PPD) is well known in haemodialysis patients (HDp). This cross-sectional controlled study was conducted to analyse the correlation of PPD response with demographical features, nutritional parameters and the distribution of peripheral blood lymphocyte (PBL) subtypes. In this study 29 HDp (17 men, 12 women; mean age 30·9 ± 9·5 years) and 13 controls (eight men, five women; mean age 29·2 ± 6·4 years) were included. The mean time spent on dialysis was 20·5 ± 17·4 months. The mean PPD response was lower in HDp than controls (7·5 ± 8 mm *vs.* 15 ± 4 mm, $P=0·001$). Fourteen patients (48%) were PPD (−) (eight men, six women; mean age 34·1 ± 11·1 years) and 15 were PPD (+) (normergic) (nine men, six women; mean age 26·8 ± 3·4 years). No difference was observed between PPD (−) and (+) groups for age, sex and time spent on dialysis. As nutritional parameters, body mass index, serum albumin, creatinine and cholesterol levels were measured and no differences were found between controls and the PPD (−) and (+) groups. Absolute lymphocyte counts were lower in HDP compared to controls (1290 ± 296 *vs.* 1570 ± 307 cells ml^{-1}; $P=0·01$). PBL subtype percentages and absolute counts (CD3, CD4, CD8, CD4/CD8, $HLADR^+CD3^+$, $CD16^+56^+$, CD19) were also similar between PPD(−) and (+) HDp.

It was concluded that PPD response cannot be predicted by the distribution of PBL subtypes. The most probable cause of this observation is regulation of PPD reactivity by *in situ* immune cells whose composition is not reflected in the distribution of PBL.

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Introduction

A decreased response to tuberculin (PPD) has been described in haemodialysis patients. An increased rate of anergy and tuberculin nonreactivity in this group of patients has been recently reported by Smirnoff *et al.* (1). Previous studies have implicated malnutrition, a common disorder in this group of patients, as a factor in tuberculin reactivity (2–4). This cross-sectional controlled study was conducted to analyse the relationship between tuberculin response and patient demographic features, nutritional parameters, and the distribution of peripheral blood lymphocyte subgroups in haemodialysis patients.

Received 4 August 1998 and accepted in revised form 23 October 1998.

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Patients and Methods

PATIENTS

In this study, 29 haemodialysis patients (17 men, 12 women; mean age 30·9 ± 9·5 years) and 13 age and sex-matched healthy controls (eight men, five women; mean age 29·2 ± 6·4 years) selected from medical staff were included. The Declaration of Helsinki's recommendations for guiding physicians in biomedical research involving human subjects were followed (5).

Exclusion criteria for this study were: history of tuberculosis or diabetes mellitus; transfusion in the last year; HIV positive; or history of a recent viral infection. Demographic characteristics of the haemodialysis patients are shown in Table 1. All haemodialysis patients were dialysed using a Cuprophan rayon dialyser (Terumo, Tokyo, Japan) three times per week using bicarbonate dialysate. Blood samples were taken just before the dialysis sessions. Body mass

0954-6111/99/020119+04 $12.00/0

TABLE 1. Demographical and nutritional parameters of the study groups

	PPD (−) HDp (n=14)	PPD (+) HDp (n=15)	Control (n=13)	Statistics
Age (years)	32·1 ± 10·1	30·6 ± 9·6	29·2 ± 6·4	n.s.*
Sex (male/female)	8/6	9/6	8/5	n.s.
Time spent on dialysis (months)	20·4 ± 17·7	20·6 ± 17·1	—	n.s.
BMI (kg m^{-2})	20·4 ± 2·5	20·2 ± 3·1	22·4 ± 2·0	n.s.
Creatine (mg dl^{-1})	9·4 ± 4·0	8·7 ± 3·6	—	n.s.
URR (%)†	58·5 ± 5·4	61·2 ± 4·8	—	n.s.
Albumin (gr dl^{-1})	4·1 ± 0·4	3·9 ± 0·4	4·0 ± 0·6	n.s.
Cholesterol (mg dl^{-1})	185·1 ± 41·7	165·4 ± 30·1	180·2 ± 36·7	n.s.

*n.s., Not significant; †URR, urea reduction ratio.

index, serum albumin, creatinine and cholesterol levels were taken as measures of nutritional status. The urea reduction ratio (URR) calculated from pre- and postdialytic blood urea nitrogen levels was taken as the index of dialysis adequacy.

TUBERCULIN TESTING

Tuberculin reactivity in haemodialysis patients was assessed by response to intradermal 5 IU PPD (Inter Vax Biologicals Limited, Canada) injected using the Mantoux technique into the volar surface of the forearm without the arteriovenous fistual. Induration was measured at 48 h. Induration of less than 5 mm was defined as PPD (−). A second tuberculin test was performed in all PPD (−) patients for a booster effect 1 week later.

PERIPHERAL BLOOD LYMPHOCYTE SUBTYPING

Immunophenotypic analysis of the cells was performed using a FACScan flow cytometer (Becton Dickinson, U.S.A.) equipped with a 15 mW air-cooled argon-ion laser. The percentages of CD3, CD4, CD8, HLADR$^+$CD3$^+$, CD16+56$^+$ and CD19 were measured using monoclonal antibodies in peripheral blood samples. A minimum of 1000 events were counted on each sample. Data analysis was performed using Lysis II software (Becton Dickinson, U.S.A.). Gating was performed using 90° right angle scatter. The fluorescence signals were amplified on a logarithmic scale.

STATISTICAL ANALYSIS

All numerical values are reported as mean ± SD. Statistical computations were done using SPSS for Windows V. 5.0 (SPSS Inc. Illinois, U.S.A.). The numerical parameters deviated from normal distribution, therefore comparisons between groups [PPD (+) haemodialysis patients, PPD (−) haemodialysis patients, and controls] were done using the nonparametric Kruskal-Wallis ANOVA test. P<0·05 was taken as the level of significance.

Results

The mean PPD response was lower in haemodialysis patients than in controls (7·5 ± 8 mm *vs.* 15 ± 4 mm; P=0·001). Fourteen patients were tuberculin-negative (48%) (eight men, six women; mean age 34·1 ± 11·1 years) and 15 (52%) were tuberculin responders (normergic) (nine men, six women, mean age 26·8 ± 3·4 years). There was no statistically significant difference in age or sex of the tuberculin responders and nonresponders. The mean time spent on dialysis was similar in responders and nonresponders; 20·6 ± 17·1 and 20·4 ± 17·7 months, respectively (P>0·05).

Body mass indexes in PPD (+) haemodialysis patients, PPD (−) haemodialysis patients and healthy controls were 20·2 ± 3·1, 20·4 ± 2·5, and 22·4 ± 2·0 kg m^{-2} respectively and did not reach statistical significance. Serum cholesterol and albumin levels were also similar across the group (Table 1).

Absolute lymphocyte count was higher in normal controls than in the PPD (+) and (−) haemodialysis groups; 1570 ± 307 *vs.* 1355 ± 250 and 1230 ± 330 cells ml^{-1} respectively (P=0·02). Comparison of peripheral blood lymphocyte subgroups (CD3, CD4, CD8, CD4/CD8, HLADR$^+$CD3$^+$, CD16$^+$56$^+$, CD19) between groups is shown in Table 2. The percentage of HLADR$^+$CD3$^+$ cells in haemodialysis patients was significantly higher compared to controls; 8·0 ± 6·1% for PPD (−) haemodialysis patients, 6·9 ± 3·9% for PPD (+) haemodialysis patients and 2·2 ± 1·8% for controls (P=0·01). The absolute count of HLADR$^+$CD3$^+$ cells was also increased in haemodialysis patients, but did not reach statistical significance. No difference in absolute count of PBL subtypes was observed between the PPD (−) and (+) groups (Table 2).

Discussion

Tuberculin anergy is common in haemodialysis patients (1,6). Malnutrition, a frequent problem in haemodialysis patients, has previously been implicated as a factor in tuberculin anergy (2–4). A recent study has shown that tuberculin anergy present at commencement of haemodialysis improves after prolonged dialysis (2) and explained

TABLE 2. Percentages of peripheral lymphocyte subsets in study groups

	PPD (−) HDp (n=14)	PPD (+) HDp (n=15)	Control (n=13)	Statistics
Lymphocytes (cells ml^{-1})	1230 ± 330	1355 ± 250	1570 ± 307†	P=0·024
CD3 %	72·1 ± 6·2	70·6 ± 8·0	65·1 ± 5·4	n.s.*
Absolute count	871 ± 238	969 ± 211	1020 ± 266	n.s.
CD4 %	39·9 ± 12·1	37·9 ± 4·9	39·0 ± 5·3	n.s.
Absolute count	483 ± 201	518 ± 16	557 ± 165	n.s.
CD8 %	30·6 ± 8·2	33·2 ± 7·1	26·6 ± 6·0	n.s.
Absolute count	356 ± 91	459 ± 144	497 ± 209	n.s.
CD4/CD8	1·4 ± 0·7	1·2 ± 0·5	1·2 ± 0·4	n.s.
HLADR$^+$CD3$^+$ %	8·0 ± 6·3	6·9 ± 3·9	2·2 ± 1·8†	P=0·01
Absolute count	98·6 ± 85·0	91·4 ± 50·5	69·6 ± 40·4	n.s.
CD56$^+$16$^+$ %	14·6 ± 4·3	16·8 ± 8·5	18·2 ± 6·6	n.s.
Absolute count	185 ± 102	219 ± 105	249 ± 150	n.s.
CD19 %	10·0 ± 4·3	9·1 ± 4·3	12·8 ± 5·3	n.s.
Absolute count	116 ± 45	128 ± 69	145 ± 64	n.s.

*n.s., Not significant; †Healthy controls different from anergic and normergic haemodialysis patients.

this observation by the improvement in appetite and less restricted diet with haemodialysis which improves nutrition (2). We were unable to show any relationship between tuberculin reactivity and duration of dialysis. This may be due to the longer time spent on dialysis in our patients (with only two patients on dialysis for less than 6 months). The lack of malnutrition in our patient group, as assessed by body mass index, serum albumin, creatinine and total cholesterol, may support the role of malnutrition in anergy. There have also been reports on the lack of a relationship between nutritional parameters and tuberculin anergy (6). In this study, we found that tuberculin reactivity was not affected by age or sex, which is consistent with the study by Smirnoff *et al.* (1).

Another factor causing tuberculin anergy may be decreased cell-mediated immunity, as has been shown in the *in vitro* models of uremic rats (7). We found in our study that the total lymphocyte count was decreased in patients on haemodialysis compared to healthy controls, as has also been observed previously (8,9). On comparing PBL subtype percentages in haemodialysis patients and controls, the only significant difference was an increase in the subgroup of HLADR$^+$CD3$^+$ lymphocytes, markers of activated T cells, in haemodialysis patients (Table 2). This supports the previous observation by Ueki *et al.* (9). The increase in the percentage of activated T cells was thought to be related to nonspecific stimulation of T cells by the dialysis membrane or other chronic antigenic stimulations. However, activated T-lymphocyte subgroup percentages were similar in PPD (−) and (+) patients in our study.

In conclusion, we believe that the tuberculin response in haemodialysis patients cannot be predicted by quantitative analysis of peripheral blood lymphocyte subtypes. The differences in tuberculin reactivity may originate from qualitative differences in lymphocyte subsets or differences in the dermal *in situ* immune response.

Acknowledgements

This study was supported by Turkish Kidney Foundation by grant number 19984.

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