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Immune defects in chronic renal impairment: evidence for defective regulation of lymphocyte response by macrophages from patients with chronic renal impairment on haemodialysis

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SUMMARY

Cellular mechanisms contributing to impaired lymphocyte proliferative responses in chronic renal impairment (CRI) were investigated using peripheral blood mononuclear cells (PBMC) from 25 patients receiving haemodialysis. Impaired T cell proliferative responses to phytohaemagglutinin were demonstrated. The hyporeactive PBMC from patients with CRI suppressed the responses of PBMC from normals to a greater degree than did control PBMC. This immunosuppression was reversed significantly by depleting adherent monocytes (MØ). To further determine if these impairments might be critically dependent on cell-cell contact, MØ from an additional 10 patients on haemodialysis were examined for ability to support B and T cell colony formation in semi-solid cultures stimulated by Staphylococcus protein A (SpA). When compared to normal controls, significantly fewer B and T cell colonies were observed with MØ from CRI patients than when autologous MØ were used. Also, T cells from patients were significantly less effective than controls in supporting B cell colony growth. Decreased T and B cell colony responses in patients were not due to a primary abnormality of these cells, since allogeneic mixing experiments showed that B and T cells from patients were able to form a sufficient number of colonies when control MØ or T cells from normals were used as accessory and helper cells. These findings suggest that although MØ-mediated suppressor activity is an important mechanism contributing to impaired lymphocyte responsiveness in patients with chronic renal impairment on haemodialysis, additional or related abnormalities in MØ 'accessory' function may also exist.

Keywords chronic renal impairment haemodialysis immune impairment

INTRODUCTION

Many complications experienced by patients with chronic renal impairment can be attributed to defects of T cell mediated immunity (Dobbelstein, 1976). The precise etiology of these defects

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remains unknown, although previous studies suggest at least three possible explanations: (a) lymphocytopaenia (Riis & Stougaard, 1959; Wilson, Kirkpatrick & Talmage, 1965; Quadracci, Ringden & Krzymanski, 1976), (b) depression of T cell function by components of uraemic serum (Elves, Israels & Collinge, 1966; Newberry & Sanford, 1971; Slavin & Fitch, 1971; Tourain *et al.*, 1975), and (c) increased suppressor-cell activity (Raskova & Morrison, 1976; Alevy, Slavin & Hutchison, 1981). In view of these findings it is possible that several different mechanisms contribute to immune impairment in these patients.

In the present study we tested the ability of peripheral blood mononuclear cell (PBMC) from patients on chronic haemodialysis to suppress the mitogenic response of normal cells and whether this suppression was mediated by adherent monocytes (MØ). Additional studies employed the focal growth of patient B or T cell colonies, aided by MØ accessory function, and stimulated by Staphylococcus protein A (SpA) in semi-solid cultures. Our results provide direct evidence that MØ suppression, as well as defective MØ accessory function, may contribute to the underlying immunological defects in patients with CRI.

MATERIALS AND METHODS

Patients. Twenty-five patients with chronic renal impairment (CRI) of various etiologies who were undergoing regular haemodialysis (4–5.5 h, three times a week) were studied. Their ages ranged from 35 to 63 years with a mean of 49 years; 17 were men and eight were women. The mean duration of haemodialysis prior to the study was 4·6 years with a range from 10 months to 13·5 years. CRI was ascribed to chronic glomerulonephritis in 15 patients and chronic pyelonephritis in three. Of the remaining seven patients, three had nephrosclerosis, two polycystic kidney disease, one had interstitial nephritis and one hereditary nephritis. Clinical details on the patients (Nos. 1–15) who provided the experimental data in Tables 1 and 2 and Figures 1 and 2 are given in Table 1, as are the details on patients (Nos. 16–25) who provided data in Figures 3–5. No patients were receiving corticosteroids or immunosuppressive drugs. None of the patients had systemic lupus or other identifiable systemic immunological disease.

All blood samples were taken prior to the commencement of haemodialysis and systemic heparinization. Twenty normal male and female donors were used as age-matched controls. In general, cells from two to four patients and two to three matched controls were assayed simultaneously.

PBMC preparations and surface markers. PBMC were obtained using standard Ficoll-hypaque gradients. The cells were washed and resuspended in RPMI 1640 supplemented with 15% heat-inactivated pooled human serum.

B lymphocytes were enumerated by the presence of surface membrane immunoglobulin (sIg) using polyvalent fluorescein-conjugated anti-human immunoglobulins (Cappel Laboratories, Fort Warington, Pennsylvania, USA). T cells were identified by the sheep red blood cell (SRBC) rosette technique. MØ were identified by their ability to phagocytose latex particles and simultaneous staining with M1 monoclonal antibody (Orthoclone, Raritan, New Jersey, USA). MØ were depleted from PBML by multiple surface adherence to plastic Petri dishes at 37°C. MØ constituted less than 5% of the non-adherent cell population, as reported previously (Bobak & Whisler, 1980).

Mitogen stimulation. Phytohaemagglutinin (PHA; Wellcome Reagents, Research Triangle, North Carolina, USA) was added in three doses known to cover the range of supra-optimal to sub-optimal mitogenic stimulation for normal cells. The final concentration of PHA in the culture wells was 10 μg/ml (high dose), 1 μg/ml (medium dose) and 0·25 μg/ml (low dose). PBMC were placed in flat-bottom microtitre plates (Linbro Plastics, Hamden, Connecticut, USA) as 10^5 viable cells per 0·2 ml in each well in triplicate. After 3 days of stimulation at 37°C in a 5% CO_2 atmopshere, the wells were pulsed for 24 h with 1 μCi of ^{3}H-thymidine, harvested by a multiple automated sample harvester and prepared for assay using liquid scintillation fluid. Stimulation due to PHA was determined by subtracting the mean ct/min obtained from triplicate control cultures.

Spontaneous suppressor assay. The spontaneous suppressor activity of freshly drawn PBMC was determined by measuring the ability of fresh mitomycin-C (m/C)-treated cells to reduce the

Table 1. Clinical data on patients with chronic renal impairment who were undergoing regular haemodialysis

Patient	Age	Sex	Race	Years on dialysis	Serum creatinine (mg %)	Cause of renal failure
1	61	M	B	5	20	hypertension-related nephropathy
2	58	M	W	3	24·5	chronic glomerulonephritis (CGN)
3	50	M	W	11	20	chronic glomerulonephritis
4	54	M	W	13·5	13·4	chronic glomerulonephritis
5	57	M	W	4	19·5	chronic glomerulonephritis
6	47	F	W	1·5	17·8	hypertension-related nephropathy
7	53	M	W	8	16·5	chronic glomerulonephritis
8	49	F	B	1·5	22	chronic glomerulonephritis
9	51	M	W	4	18·3	polycystic kidney disease
10	38	M	W	5	24	interstitial nephritis
11	56	M	W	1	15·7	hypertension-related nephropathy
12	44	M	W	3	21·15	chronic glomerulonephritis
13	48	M	B	3	10	chronic glomerulonephritis
14	47	M	W	13	19·4	chronic glomerulonephritis
15	32	M	B	2	19·6	chronic glomerulonephritis
16	40	M	W	3	12·3	chronic glomerulonephritis
17	35	F	W	2	13·7	chronic glomerulonephritis
18	47	M	W	4	15·6	polycystic kidney disease
19	51	F	W	3·5	11·8	chronic pyelonephritis
20	56	F	W	5	21·0	chronic pyelonephritis
21	51	M	W	5	16·2	chronic glomerulonephritis
22	30	F	B	4	9·6	hereditary nephritis
23	48	F	B	0·83	10·5	chronic pyelonephritis
24	49	F	W	3	15·2	chronic glomerulonephritis
25	31	M	W	4	11·5	chronic glomerulonephritis

miogenic response to PHA of normal cells (Dwyer & Johnson, 1982). Fresh indicator cells from normal individuals were co-cultured with equal numbers of m/C-treated PBMC from chronic haemodialysis patients or normal subjects. After a 72-h exposure to the three doses of PHA, all cultures were pulsed with ^{3}H-thymidine and processed as described above. Results are expressed both as the mean ct/min obtained from triplicate co-cultures ± 1 s.d., and as percentage suppression. Percentage suppression was calculated by comparing the response of indicator cells, cultured with control or m/C-treated cells from patients, with the responses obtained when indicator cells were cultured alone.

Proliferative and spontaneous suppressor cell assays were repeated after 2–6 weeks in several patients. Similar results were found in repeat studies.

Cell preparation for semi-solid microcolony cultures. PBMC were depleted of MØ by surface adherence. B and T cell enriched populations were obtained by rosetting MØ-depleted PBMC with neuraminidase-treated SRBC followed by centrifugation over a Ficoll-hypaque gradient. The T-depleted, B-lymphocyte-enriched suspensions routinely contained 65–80% sIg^+ cells and less than 10% contaminating MØ. Less than 3% contaminating MØ were present in the T cell enriched population among which 96% of the cells re-rosetted with SRBC. More than 90% of the cells adhering to plastic Petri dishes after rinsing with media were MØ. Subsequently these cells were removed with a rubber policeman. Details of these techniques have been reported previously (Bodak & Whisler, 1980; Dwyer & Johnson, 1982; Whisler, Bodak & Newhouse, 1981).

Semisolid colony cultures. Semi-solid agarose microcultures were performed in flat-bottomed microwell plates (No. 3040, Falcon Plastics, Oxnard, California, USA). B cell colony formation was studied by using the single- and two-layer (liquid–semi-solid) techniques as described previously

(Bodak & Whisler, 1980; Whisler *et al.*, 1981). In the single-layer system, 5×10^4 B-enriched cells were supplemented with 2×10^4 MØ in 0·1 ml. In the dual-liquid–semi-solid system, $2{\cdot}5 \times 10^4$ B-enriched cells were placed in 0·1 ml semi-solid media and after solidification were overlayed with 0·1 ml of T-cells at 10^6/ml.

T-lymphocyte colony formation was studied by the single-layer technique employing 5×10^4 T cells (Tsakalos *et al.*, 1984). Accessory-cell function was assayed with 2×10^4 MØ per well. All cultures were stimulated with 10 μg/ml SpA (Pharmacia, Piscataway, New Jersey, USA). Colonies (distinct aggregates consisting of 15 or more cells using a Zeiss 135 inverted phase contrast microscope) were counted after incubation for 7 days at 37°C in a humidified atmosphere of 95% air, 5% CO_2. The number of colonies detected was expressed as the mean ± s.e. of triplicate samples.

Identification of cells derived from the colonies. The percentage of colonies expressing sIg^+ or cytoplasmic immunoglobulin (cIg^+) was determined *in situ* by overlaying monolayer cultures with an appropriate dilution of rabbit anti-human k or λ $F(ab')_2$ fragments. An equivalent concentration of fluoroscein-conjugated IgG from normal rabbit serum was used as control. After incubation for 30 min a 4°C, the cultures were rinsed repeatedly with medium until background fluorescence was negligible. Colonies containing cells positive for sIg and cIg were enumerated with a Zeiss 135 epifluorescent microscope. T cell colonies were similarly characterized *in situ* by overlaying with dilutions of mouse anti-human T3 monoclonal antibody (Orthoclone). After incubation for 1 h at 24°C, colonies were treated with a rabbit anti-mouse antibody (1:30 dilution) and incubated for an additional 1 h at 24°C. Then cultures were rinsed three times with PBS and treated with a 1:30 dilution of fluorescein-conjugated goat anti-rabbit antibody for 1 h at 24°C. No sIg^+ cells were observed on examination of the T cell colonies. Pretreatment of cells prior to culture with anti-T3 antibody + complement, completely ablated colony formation.

Statistical analysis. The significance of the differences between means was evaluated by the paired and unpaired Student's *t*-test for paired and unpaired observations respectively.

RESULTS

Characteristics of isolated PBMC suspensions

Haemodialysis patients had a higher percentage of monocytes compared with normal controls (Table 2). Ten of the 15 patients were lymphopaenic (< 1500 WBC/mm^3).

Mitogenic response before and after removal of adherent cells

PBMC from haemodialysis patients showed significantly lower responses to the middle and high doses of PHA when compared with responses of matched controls (Fig. 1). Removal of MØ from

Table 2. Cellular composition of PBMC suspensions isolated over Ficoll-hypaque gradients in patients on chronic haemodialysis and in controls

Group	T cells (SRBC rosette$^+$)	B cells (sIg^+)	MØ (Latex$^+$ + M1$^+$)	Ratio T:MØ
Patients ($n=15$)	1377 ± 145* (64 ± 1·5†)‡	273 ± 29 (13·3 ± 0·8)	479 ± 46 (22·7 ± 1)	2·9 ± 0·7
Controls ($n=10$)	1580 ± 80 (70·5 ± 1·5)	325 ± 14 (15·3 ± 0·5)	380 ± 46 (14·4 ± 1·5)	5·6 ± 0·8
P value	N.S.	N.S.	< 0·05	< 0·01

* Mean absolute number of cells per μl ± s.e.

† Mean percentage of total mononuclear cells ± s.e.

‡ Numbers in parenthesis are the percentage total cells ± s.e.

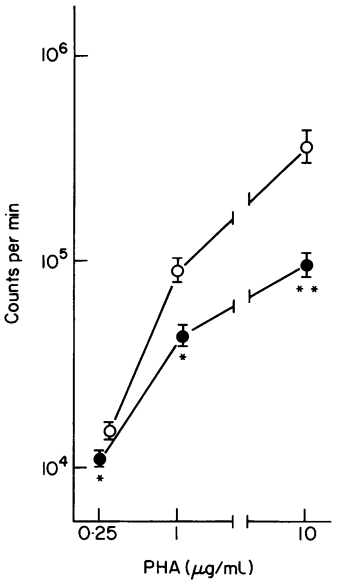

Fig. 1. Dose response curve for PHA stimulation of PBMC from control ($n = 10$, ○) and patients ($n = 15$, ●) with chronic renal impairment on regular haemodialysis. The results are expressed as mean ± s.e. * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

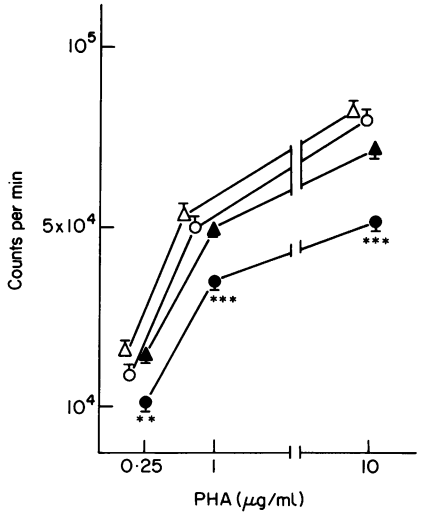

Fig. 2. Response to PHA stimulation of PBMC from normal individuals (○, △) and patients on chronic haemodialysis (●, ▲) before (○, ●) and after (△, ▲) the removal of adherent cells. Bars indicate mean ± s.e. (normals = 10; patients = 15). *$P < 0{\cdot}01$, ** $P < 0{\cdot}001$.

PBMC preparations resulted in a significant enhancement of the mitogenic response in the patients, but not the controls (Fig. 2).

Spontaneous suppressor-cell activity of freshly drawn PBMC before and after the removal of adherent cells

Mixing experiments were performed to examine further the hyporesponsiveness to mitogens of cells from patients and explore the possibility that MØ were mediating suppression. Table 3 shows that

Table 3. Spontaneous suppressor activity of freshly drawn PBMC from chronic haemodialysis patients and controls before and after removal of adherent cells

Control PBMC responder cells (10^5)	Mitomycin-C treated cells (10^5)	No. of experiments	PHA dose ct/min, mean ±s.d. 0·25 μg/ml	1 μg/ml	10 μg/ml
+	–		16,675±3,750 (0)*	48,650±3,350 (0)	73,050±7,700 (0)
+	Control	8	13,440±2,350 (19)	45,050±4,480 (7)	65,030±5,200 (11)
+	Patient	15	8,400±11,450 (50)	34,170±2,600 (30)†	51,700±5,300 (29)†
+	Control non-adherent	8	17,200±3,410 (−3)‡	50,060±4,900 (−3)	68,500±1,500 (−6)
+	Patient non-adherent	15	13,900±2,980 (17)	48,550±4,100 (1)	66,030±4,800 (9)§

* Numbers in parentheses represent percentage suppression.

† The responses by control PBMC obtained in the presence of patient cells were significantly different from the response obtained in the presence of normal control cells at $P<0·05$.

‡ Negative values indicate a lack of suppression.

§ All of the responses of control PBMC obtained in the presence of patient non-adherent cells were significantly different from the response obtained in the presence of patient PBMC ($P<0·01$ (0·25 μg/ml); $P<0·001$ (1 and 10 μg/ml).

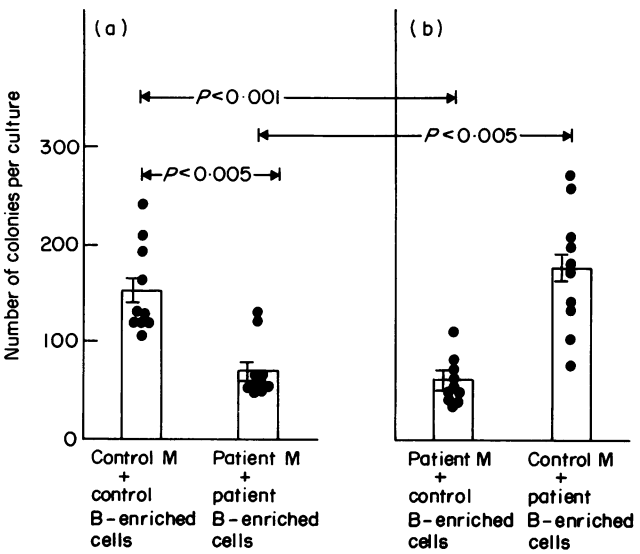

Fig. 3. The effects of MØ from normal control individuals or patients on Staphylococcus protein A (SpA)-induced formation of colonies of non-T cells (B-enriched cells) in single-layer cultures. Control B-enriched cells were seeded with control (autologous cell mixtures, a) or patient (allogeneic cell mixtures, b) MØ, and patient B-enriched cells were seeded with patient (autologous, a) or control (allogeneic, b) MØ. Bars show the mean ±s.e. of triplicate cultures counted on day 7 of culture. The responses of allogeneic combinations of control MØ and B-enriched cells from normals were not significantly different from those obtained with autologous combinations.

the response of normal PBMC co-cultured with (allogeneic) m/C-treated control cells was superior to the response of these cells co-cultured with m/C treated cells from patients and that removal of adherent cells reversed this effect.

Defective MØ support of B cell colony responses in CRI patients

The hyporeactive cells of patients failed to respond normally after monocyte depletion. On the other hand, increased suppressor cell activity by the PBMC of patients was abolished after elimination of adherent cells. These two findings suggest that in addition to active suppression by the MØ from patients with CRI there exists an additional intrinsic defect among their PBMC. In order to further characterize this defect, B and T cells from 10 additional patients undergoing haemodialysis were examined for their ability to form colonies in semi-solid cultures with the help of accessory function of MØ. Fig. 3a shows that patient B cells plus autologous MØ formed significantly fewer colonies than did controls. In allogeneic mixing experiments (Fig. 3b), normal B-enriched cells supported by MØ from patients with CRI formed significantly fewer colonies than with autologous MØ. In contrast, colony responses of B cells from patients supported by normal MØ were 2·2-fold greater than those containing autologous MØ. Less than 10 colonies were observed for B cells from both patients and control subjects stimulated with SpA in the absence of any MØ. The colony responses obtained with allogeneic combinations of cells from normals were no different than those observed with the autologous cells of normal subjects.

B cell colony responses supported by T cells in normal subjects and haemodialysis patients

The above data suggested an impaired MØ accessory cell function rather than an intrinsic B cell abnormality. We next attempted to determine whether B cells from patients could respond to normal T cell help (Whisler *et al.*, 1981; Kumagai *et al.*, 1982). Fig. 4a shows that T cells from patients were less effective than normal T cells in promoting the colony growth of autologous B cells and of allogeneic B cells from normal subjects. In contrast, the colony responses of the patient's B cells increased substantially when allogeneic normal T cells, rather than autologous T cells were used as source of help. Allogeneic T cells from normals supported B cell colony responses equivalent to autologous T cells from normal subjects.

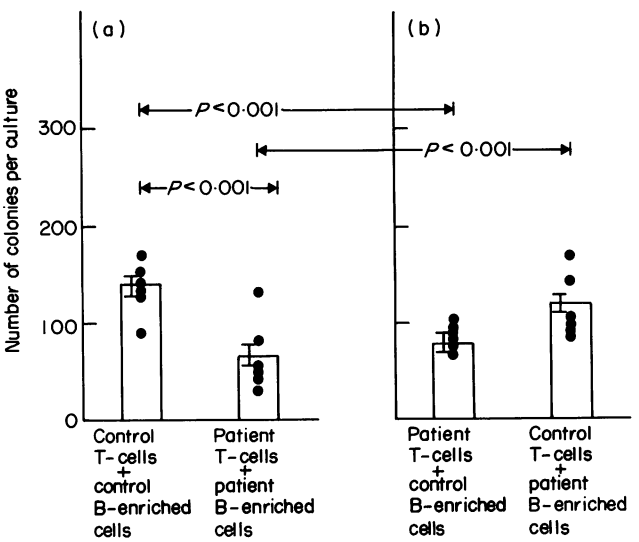

Fig. 4. The effects of T cells from normal individuals or haemodialysis patients on the formation of colonies by non-T cells (B-enriched cells) seeded in the agarose lower layer. T cells were in the upper liquid layer and were stimulated by SpA. Bars show the mean ±s.e. of triplicate cultures counted on day 7 of culture. (a) Autologous cell mixtures; (b) Allogeneic cell mixtures.

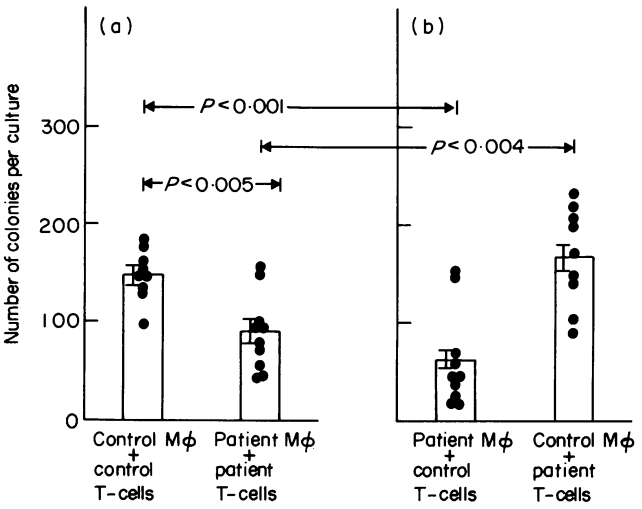

Fig. 5. The effects of MØ from normal individuals or haemodialysis patients on the SpA-induced formation of colonies of T cells in single-layer cultures. Normal T cells were seeded with normal (autologous cell mixtures, a) or patient (allogeneic cell mixtures, b) MØ. Patient T cells were seeded with patient (autologous, a) or normal allogeneic, b) MØ. Bars show the mean ±s.e. of triplicate cultures counted on day 7 of culture.

Colony-forming ability of T lymphocytes from normal individuals and patients

Since the failure of T cells from patients to generate the 'required' helper activity might have arisen from an inability of these cells to be fully activated and respond to the SpA stimulus, we evaluated the colony-forming capacity of T cells stimulated by SpA (Sakane & Green, 1978; Shibasaki *et al.*, 1978), which requires MØ for optimal responses (Kumagai *et al.*, 1982; Tsakalos *et al.*, 1984). Fig. 5a shows that the colony responses of T-cells from haemodialysis patients supplemented with autologous MØ, were about 40% less than controls. Similarly, MØ from patients were significantly less effective than autologous MØ in supporting colony growth of T cells from normal controls (Fig. 5b). On the other hand, the addition of normal allogeneic MØ to the cultures of T cells from patients resulted in about a doubling of their colony responsiveness. Control experiments performed with allogeneic and autologous combinations of cells from normal subjects failed to demonstrate any significant differences.

DISCUSSION

Impaired proliferative reponses to mitogens by PBMC from CRI patients has been demonstrated for more than a decade but the responsible mechanisms are still not well understood. Alevy *et al.* (1981) and Alevy & Slavin (1981) have shown, using spleen cells from uraemic rats, that the impaired response to mitogens is due to adherent cells with suppressor function. In the present study we have extended these observations to humans. The PBMC from most patients displayed impaired responses following stimulation with PHA. These lower responses were improved after the removal of adherent cells. Monocytes from patients with CRI might be more potent suppressors relative to normal monocytes, since their elimination resulted in a significant enhanced mitogenic response of the remaining non-adherent cells. The latter possibility was supported by findings in spontaneous suppressor-cell co-culture experiments in which the hyporeactive PBMC from patients were more suppressive of the mitogenic response of normal cells than were the PBMC from healthy controls. Increased suppressor activity of the PBMC from patients was largely abrogated after elimination of adherent cells.

Monocyte mediated suppression of immune responses has been reported in a wide variety of human diseases including tumours of lympho-reticular origin such as Hodgkin's disease (Schecter

& Soehnlen, 1978), solid tumours such as lung cancer (Zembala *et al.*, 1977), chronic tuberculosis (Schecter & Soehnlen, 1976), disseminated fungal diseases (Stobo, 1977), sarcoidosis (Katz & Fauci, 1978), and autoimmune disease such as systemic lupus erythematosus (Markenson, Morgan & Lockshin, 1978). Spontaneously active suppressor cells can also be T cells (Bresnihan & Jasin, 1977). However, the abrogation of suppressor activity after the elimination of adherent cells in the allogeneic cultures is against this possibility, but not entirely since adherent human suppressor T cells have been reported (Dwyer & Johnson, 1981).

MØ from patients with CRI were also evaluated for the ability to support the focal growth of human B or T cell colonies stimulated by SpA. The results from these experiments confirm the observations of others (Radnay, Goldman & Rosenszajn, 1979; Muraguchi *et al.*, 1980; Tsakalos *et al.*, 1984) that the majority of cells forming these colonies display B or T cell characteristics depending on the populations used. We found that MØ from patients were inferior to normal MØ in supplying the necessary accessory function to promote B or T cell colony responses. It is unlikely that defects in cellular functions that require cell–cell contact explain the MØ defects, since colony responses in semi-solid media minimize the cell–cell contact present in liquid cultures.

Further work is needed to see if the functional defects of MØ from patients represents an inability to secrete lympho-stimulatory molecules such as Interleukin-1, the prototypic monokine that promotes T cell activation, or B cell colony stimulating or growth factors. We do not think that elaboration of prostaglandins is responsible for the MØ defects of CRI patients since treatment *in vitro* with indomethacin does not reverse the defects. The inability of monocytes to optimally promote T cell colony responses may result from the presence of monocytes that only express HLA-DR weakly (Tsakalos *et al.*, 1984). Alternatively, monocytes from patients with chronic renal failure may process or present SpA inadequately. The latter may be associated with the metabolic disturbances present in CRI such as hyperammonaemia (Scala & Oppenheim, 1983) and/or hyperhistaminaemia (Beer *et al.*, 1982), but since the defects are still present in well-controlled patients on chronic haemodialysis, the causes may be due to additional factors such as exposure of cells to extra-corporial circuits and/or effects of frequent blood transfusions. Understanding the MØ defects that alter cell-mediated immunity in chronic renal impairment is essential if corrective therapy is to be approached.

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