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Monocyte disorder causing cellular immunodeficiency: a family study

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SUMMARY

We report a familial type of monocyte dysfunction not recognized previously. This disorder was observed in a young adult man with a long clinical history of recurrent, self-limited episodes of cryptogenic fever accompanied by digestive and respiratory symptoms and repeated oral and skin infections. Lectin-induced lymphocyte transformation was reduced and skin tests revealed anergy to tuberculin and candidin. Monocytes from this patient exhibited markedly diminished expression of cytoskeletal vimentin intermediate filaments, HLA-DR antigens and immunological receptors for IgG Fc and C3b. These abnormal monocytes demonstrated impaired phagocytosis and reduced accessory cell function on PHA-mediated lymphocyte activation. Release of soluble lymphocyte-activating factors by these cells was found to be defective. Lymphocytes from the patient responded appropriately to lectin in the presence of normal monocytes. Two family members of the proband presented similar monocyte defects although they only manifested minor clinical symptoms. This syndrome underlines the interest of testing monocyte markers and function in subjects with clinical manifestations of immunodeficiency.

Keywords monocyte function familial immunodeficiency intermediate filaments fever

INTRODUCTION

Cells of the monocyte–macrophage lineage, in addition to their phagocytic function, exert bi-directional interactions with lymphocytes that are important in the development of the immune response (Unanue & Allen, 1987; Johnston, 1988). Expression on the monocyte membrane of immunological receptors for the Fc portion of IgG (FcR) and C3b (CR1) serve to bind opsonized particles (Griffin, 1982) while expression of HLA-DR molecules is crucial for antigen presentation to T cells (Beller, 1984; Unanue & Allen, 1987). Activated monocytes and macrophages release interleukin-1 (IL-1) that stimulates T lymphocytes to secrete interleukin-2 (IL-2) which amplifies T cell blastogenesis (Maizel *et al.*, 1981; Palacios 1982; Chu *et al.*, 1984; Unanue & Allen, 1987).

Despite the critical role of mononuclear phagocytes as accessory cells in the immune response, only isolated reports have related intrinsic monocyte defects to clinical states of immunodeficiency (Snyderman *et al.*, 1973). Here we describe a cellular immunodeficiency syndrome associated with a monocyte abnormality characterized by decreased phagocytic activity, reduced display of immunological surface receptors (CR1, FcR and DR) and diminution in the expression of cytoskeletal vimentin filaments (VF).

Changes in monocyte cytoskeleton have been described in a variety of clinical entities such as viral hepatitis or chronic fatigue syndrome (Prieto *et al.*, 1989a,b). In these cases deficient monocyte expression of both VF and immunological membrane receptors was corrected *in vitro* by incubation with interferon-gamma (IFN-γ) (Prieto *et al.*, 1989a) or naloxone (Prieto *et al.*, 1989b). In the family described here, these agents were not able to revert the monocyte changes. In addition, in this family, the abnormal monocytes were responsible for impaired cellular immune reactivity as they failed to induce proliferation of both autologous and homologous lymphocytes.

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CASE REPORT

The patient, a 32-year-old caucasian man, presented a long history, dating from infancy, of repeated and self-limited episodes of fever of unknown origin lasting 3 to 10 days and recurring 8 to 12 times per year. They were usually preceded by throat ache, cough and sputum and accompanied by erythematous skin rash, myalgias, abdominal pain and constipation followed by diarrhoea when fever subsided. Other clinical features included prolonged febrile reactions to vaccines, delayed wound healing, episodes of conjunctivitis and oral candidiasis, occasional vesiculous mucosal lesions in the mouth and cutaneous abscesses in extremities which have healed leaving scars.

Laboratory studies during apirexial periods showed a mean white blood cell count of 7000/mm^3 with 1% band, 61% neutrophils, 2% eosinophils, 34% lymphocytes and 2% monocytes. Routine laboratory tests were normal including immuno-

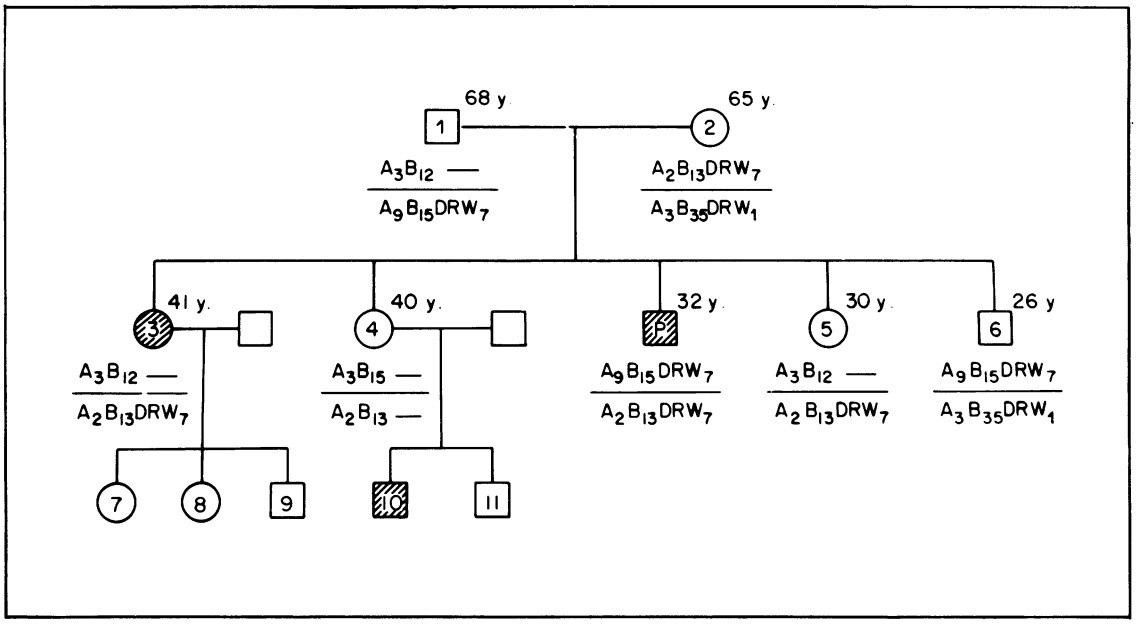

Fig. 1. Pedigree of the family (squares, men; circles, women) manifesting monocyte dysfunction. Shadowed cases represent affected members of the family. The proband is denoted by P. Age and HLA phenotype are depicted.

globulins, total complement and fractions and neutrophil NBT test. During the febrile episodes leucocytosis, neutrophilia and increased alfa-2-globulin was observed. During these episodes all bacteriological studies were negative. Chest X-ray films, bronchography, barium meal and enema, IV-urography and hepatobiliary ultrasonography did not manifest abnormal findings. Antibodies against deficiency virus HIV-1 and cytomegalovirus (CMV) were negative; anti-Epstein–Barr virus (EBV) capsid antigen were positive (1:640 titre) but negative against EBV-early antigen ($< 1:10$).

Skin testing showed anergy to candidin and tuberculin but a positive response to dinitrochlorobenzene was obtained after sensitization. As exposed below, blood lymphocytes showed low proliferative response to phytohaemagglutinin (PHA) and further investigation of the cellular immune deficiency exhibited by the patient revealed an abnormality of monocytes. Family studies disclosed similar monocyte defects in a sister and a nephew (no. 3 and 10 in the pedigree; Fig. 1). The sister had a long history of marked astenia and the nephew presented frequent but not serious respiratory infections. Except for the

Table 1. Monocyte markers and function in controls (C), in the proband (P) and his family

	Sheep erythrocyte rosseting (%)			Markers detected by monoclonal antibodies (%)			Phagocytosis indices	
Case	E	EA	EAC	Leu-M3	VF	DR	*Candida albicans*	Latex
C ($n=6$)	1–3[c]	59–79	62–80	92–95	64–72	64–87	60–91	62–89
P*	2	5	7	91	ND	4	15	ND
	2	12	13	92	7	16	ND	12
3*	4	14	13	91	ND	30	18	ND
	3	12	25	92	9	21	ND	20
5	1	59	63	93	ND	87	70	ND
7	3	69	65	94	ND	83	68	ND
10*	2	14	15	93	ND	12	27	ND
	3	19	32	90	5	20	ND	18
11	3	65	67	92	ND	75	60	ND

Case numbers are as in Fig. 1.
For controls, range is shown. VF, cytoskeletal vimentin intermediate filaments; ND, not done.
In cases P, 3 and 10 two studies were performed on different days.
* Abnormal results.

Table 2. Surface markers of peripheral blood mononuclear cells in controls (C), in the proband (P) and his family

Case	Positive cells (%)							
	CD2	CD5	CD4	CD8	DR	sIg	Leu-M3	CD4/ CD8 index
C ($n=6$)	72–82	65–78	30–45	19–32	18–30	15–25	13–20	1·3–2·1
P (1984)	59	ND	38	26	ND	20	ND	1·46
(1984)	60	ND	24	16	ND	14	14	1·50
(1984)	62	ND	17	19	34	ND	13	0·89
(1985)	52	ND	24	21	16	20	13	1·14
(1987)	58	60	21	24	16	ND	14	0·87
1	72	ND	36	32	19	ND	22	1·15
2	79	ND	32	27	20	ND	27	1·18
3 (1984)	82	ND	14	44	19	ND	10	0·31
(1987)	75	72	25	17	14	ND	8	1·47
5	81	ND	38	31	21	ND	18	1·22
10	64	61	37	18	12	ND	10	2·05

Case numbers are as in Fig. 1.
For controls, range is shown.
sIg, surface immunoglobulin; ND, not done.
* Affected members.

Table 3. Mitogenic response to PHA (2·5 μg) in cultures of lymphoid cells (1×10^6 cells) recombined with autologous or homologous monocytes ($2{\cdot}5 \times 10^5$ cells)

	Monocytes		
	Patient	Sister 3	Sister 5
Lymphoid cells			
Patient	18,512	16,724	64,512
Sister 3	15,436	16,712	74,512
Sister 5	18,714	25,714	114,412

Peripheral mononuclear cells were separated into adherent and non-adherent cells by allowing monocytes to adhere to plastic culture dishes. Then, cultures of lymphoid cells reconstituted with autologous or homologous monocytes were performed in the presence of PHA as expressed in the table.

Sister 3 was affected by similar cellular immune deficiency to that found in the patient. Cellular immunity was normal in sister 5. Both sisters were HLA identical and shared one haplotype with the patient.

PHA, phytohaemagglutinin.

finding under the electron microscope of a poorly developed cytoskeleton, no other significant morphological changes were detected in the patient's monocytes.

MATERIALS AND METHODS

Phenotypic characterization of peripheral blood mononuclear cells (PBMC)

A standard direct immunofluorescence (DIF) technique was done using monoclonal antibodies against specific membrane markers. The following monoclonal antibodies were used: anti-CD2 (DAKO CD2, Dakopatts), anti-CD3 (OKT3, Ortho), and anti-CD5 (leu-1, Becton Dickinson) as markers of T lymphocytes, anti-CD22 (DAKO CD22, Dakopatts) as marker of B lymphocytes, and the percentage of monocytes was evaluated using Leu M3 (Becton Dickinson). HLA phenotype was studied by microcytotoxicity assay (Mittal *et al.*, 1968).

Lymphocyte transformation with PHA

Lectin-induced blastogenesis was studied by culturing PBMC in the presence of 2·5 $\mu g \times 10^6$ cells of PHA (Difco, Detroit, MI). Cells were grown in a humidified CO_2 incubator at 37°C for 72 h. Twenty-four hours before harvesting they were pulsed with 0·5 μCi of ^{3}H-thymidine (Amersham, Artington Heights, UK). Then they were collected on a microwell harvester and the radioactivity counted.

Monocyte studies

Monocytes were purified according to Broff, Jonsen & Geha (1981). Membrane markers, VF expression and phagocytic function were analysed as previously described (Prieto *et al.*, 1989a,b). Briefly, the expression of VF by fixed monocytes was studied by indirect immunofluorescence technique using monoclonal anti-vimentin antibodies (Dakovimentin M. 725; Dakopatts) and FITC-conjugated goat anti-mouse antisera. The percentage of monocytes expressing VF was assessed using a fluorescence microscope. Two-hundred cells were counted in every assay. CR1 and FcR were analysed by EAC and EA rosetting of purified monocytes, respectively. The expression of HLA-DR molecules was studied by DIF using OKla-1 monoclonal antibody (Ortho). The phagocytic function of monocytes was assessed by ingestion of latex particles and *Candida albicans*. The percentage that ingested yearst of latex particles was the phagocytosis index. All the above experiments were also performed after overnight incubation of monocytes with IFN-γ (Meloy, Springfield, VA) or with naloxone (Abelló, Madrid, Spain).

Mixed cultures of adherent (monocyte-enriched) and non-adherent lymphoid PBMC

Separation of cell populations. This procedure was done according to Broff *et al.* (1981). Briefly, isolated PBMC were washed with HBSS and suspended as a concentration of 5×10^6 cells/ml in enriched RPMI (RPMI-1640 (Difco) with 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 U penicillin/ml and 50 μg/ml streptomycine. Cell suspensions were incubated for 1 h in 100 × 15 mm Petri dishes (Falcon, Oxnard, CA) at a volumen of 10 ml/dish at 37°C in an atmosphere of humidified CO_2. Non-adherent cells were collected, washed three times with PBS and resuspended in enriched RPMI at 1×10^6 cells/ml. Adherent cells were recovered from the dishes after overnight incubation in the same medium. The dishes were washed three times with PBS, incubated for 10 min in PBS at 4°C and scraped off gently with a cell scraper. Cells were washed twice in HBSS and resuspended in enriched RPMI. The viability exceded 95% and more than 90% of cells were esterase positive. In these adherent-cell suspensions the percentage of positive cells with leu-M3 antibody was greater than 90%.

Cell cultures. Cells were routinely cultured in microtitre plates (Falcon) in triplicate wells. Blastogenesis was induced using 2·5 μg/ml PHA in cultures (1 ml) of non-adherent lymphoid cells (1×10^6/ml) from both patients and controls in the presence of variable amounts ($1{\cdot}5 \times 10^5$, $2{\cdot}5 \times 10^5$ and 5×10^5 cells/ml) of autologous or homologous monocytes.

To estimate the production of IL-1 we analysed the mitogenic response of non-adherent lymphoid cells to PHA (2·5 μg/10^6 cells) in the presence of different final dilutions (1:2, 1:4, 1:8, 1:16) of conditioned medium obtained from autologous or homologous monocytes. Conditioned medium was obtained according to Toribio, de Landazuri & Lopez-Botet (1983) by culturing 2×10^6 adherent cells in 60-mm plates (Falcon) in 2 ml of fresh MEM supplemented with 10% FCS to which 5 μg of lipopolysaccharide from *Escherichia coli* (LPS, 055BS, Difco) were added. After incubation for 24 h at 37°C in an atmosphere of humidified 5% CO_2, supernatants were centrifuged at 600 *g* for 15 min and assayed for lectin-augmenting activity in cultures of non-adherent lymphoid cells.

Viral markers

Anti-HIV-1 and anti-CMV were determined by enzyme immunoassay (Abbot Laboratories, N. Chicago, IL and Whittaker Bioproducts, Walkerswille, MD, respectively). Anti-EBV (EBV test, Gull Laboratories, Salt Lake City, UT) was analysed by standard immunoflorescence technique.

RESULTS

Monocytes from the proband and from family members 3 and 10 (Fig. 1) demonstrated a decreased expression of VF, DR, FcR and CR1 and diminished phagocytosis indices (Table 1). Incubation of monocytes with IFN-γ or naloxone did not modify monocyte markers and function in the affected subjects (data not shown).

In the study of PBMC subsets (Table 2) no significant alterations were observed in the number of monocytes, T and B lymphocytes in the patient and his family. However, a reduced number of CD4-positive cells was occasionally found in both the patient and sister 3. PBMC expressed normally both DR molecules (Table 2) and Fc receptors (21% in the patient and 19% in his affected sister).

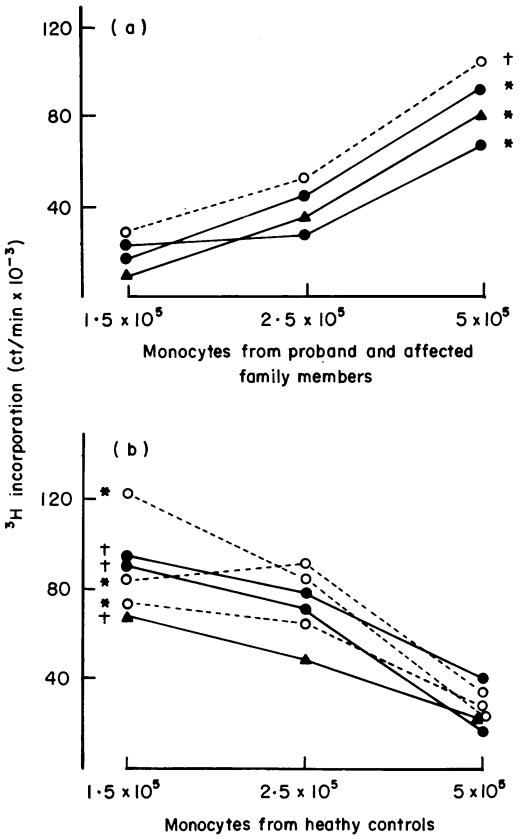

Fig. 2. Phytohaemagglutinin (PHA) (2·5 μg) induced blastogenesis of lymphocytes (1×10^6 cells) from proband (▲), affected family members (●) and healthy controls (○) reconstituted with variable amounts of monocytes from proband and affected family members (a) and from non-affected family members and non-related healthy controls (b). * Autologous and † homologous monocytes.

Lymphocyte transformation with PHA was diminished in the affected subjects but was similar to healthy controls in the other members of the family (data not shown). Mixed cultures of adherent and non-adherent cells (Table 3) showed that monocytes from the proband and the affected family members were responsible for decreased lymphocyte blastogenesis. Lymphocytes from the proband and sister 3 replicate properly with normal monocytes. On the other hand lymphocytes from sister 5 (healthy member of the family) manifested a low response to PHA when incubated with monocytes from either the patient or sister 3 (affected family member) (Table 3).

Furthermore, Fig. 2 shows that lymphocytes both from healthy subjects and individuals with the immune disturbance proliferate more intensely when concentrations of monocytes from the latter were increased (Fig. 2a) and less when the proportion of monocytes from healthy controls was augmented (Fig. 2b).

Finally, whereas LPS-stimulated monocyte-derived medium from healthy subjects induced proliferation both in the proband and in controls (Fig. 3b), the monocyte-derived medium from individuals with monocyte dysfunction was not able to produce lymphocyte blastogenesis in the proband or in healthy subjects (Fig. 3a).

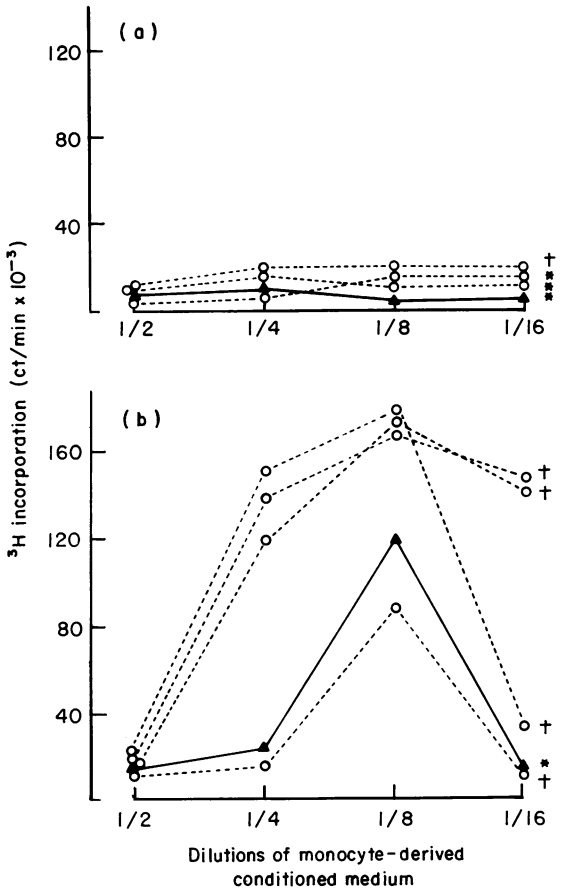

Fig. 3. Phytohaemagglutinin (PHA) (2·5 μg) induced blastogenesis of lymphocytes from the proband (▲) and healthy controls (○) in the presence of increasing dilutions of monocyte derived conditioned medium obtained from the patient or * affected family members (a) and from † non-affected family members or healthy controls (b). Monocyte-derived conditioned medium was obtained by treating peripheral monocytes (2×10^6 cells) with *E. coli* lipopolysaccharide (5 μg).

DISCUSSION

We have described a familial monocyte defect associated with a clinical syndrome of cellular immunodeficiency. Peculiar to the disorder is the very poor expression of cytoskeletal intermediate VF in monocytes, demonstrated both electronmicroscopically and by immunofluorescence staining. Monocyte intermediate filaments are quite dynamic structures positively modulated by cytokines such as IFN-γ (Prieto *et al.*, 1989a) and negatively by hormones such as endorphins or enkephalins (Prieto *et al.*, 1989b,c). However, neither IFN-γ nor the opioid antagonist naloxone were able to stimulate VF expression in the abnormal monocytes of the family here reported. Changes in monocyte cytoskeleton organization were accompanied by marked reduction in both phagocytic activity and surface display of functional membrane markers including FcR, CR1 and DR. It seems plausible that these defects may stem from disturbed cytoskeletal function since VF anchor to membrane molecules and regulate membrane processes (Geiger, 1987).

Monocytes are known to play a dual cooperative and suppressor role in T cell activation through the production of lymphocyte-activating factors, mainly IL-1, and the synthesis of suppressor mediators such as PGE_2 (Khansari, Chou & Fundenberg, 1985; Unanue & Allen, 1987). Although lymphocyte transformation was low in the proband and affected family members, no detectable intrinsic lymphocyte defects were found since in these cases lymphocyte expression of surface receptors was noted and blastogenesis normalized when lymphocytes were co-cultured with monocytes from healthy individuals. However, isolated adherent cells from subjects with monocyte disorder failed both to cooperate in lymphocyte proliferation and to release soluble lymphocyte-activating factors. Additionally, in co-cultures of lymphocytes with varying amounts of monocytes, we observed that high proportions of normal monocytes induced a reduction in lymphocyte transformation, while the opposite was observed when monocytes were taken from the proband or affected family members. Thus, it appears that in this syndrome there is a diminution in the number of monocytes functionally able to cooperate in lymphocyte activation and that this may be at the root of the cutaneous anergy to candidin and tuberculin present in our patient.

Monocytes are an important source of endogenous pirogens. It may seem surprising that frequent episodes of fever are a prominent clinical feature in a patient such as ours, in whom there is evidence of impaired monocyte function. This apparent paradox may be explained by the action of endogenous pirogens other than IL-1 such as tumour necrosis factor and interferons (Dinarello, 1986). In the synthesis of these mediators not only monocytes participate but also other cells such as lymphocytes and vascular smooth muscle cells (Warner & Libby, 1989).

The familial presentation of the monocyte disorder here described suggests a genetic basis for this disease. In this respect it should be noted that no clear linkage to the HLA region was present. However, since monocytes can be persistently infected by viruses (Morahan, Connor & Leary, 1985; Rouse & Horohow, 1986) which may induce functional changes in these cells (Morahan *et al.*, 1985; Rouse & Horohow, 1986), the monocyte disorder we described may represent the intrafamilial spreading of a viral infection. Further studies are needed to ascertain the genetic or adquired nature of this disease.

We describe a familial disorder characterized by poor expression of VF, reduced membrane display of CR1, FcR and DR and defective accessory/cell function. This disorder illuminates the role of monocytes in the integrated function of the immune system and underlines the interest of testing monocyte markers and function in subjects suspect of immune dysfunction.

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