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Enhanced formation of giant cells in common variable immunodeficiency: Relation to granulomatous disease

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ABSTRACT

Peripheral monocytes from patients with common variable immunodeficiency (CVID) had on average a 2 fold greater tendency to form giant cells in medium without additional cytokines. Giant cell formation was faster and 3 to 5 fold higher in most CVID cells compared to normal. Addition of IL4, GMCSF, IFNγ, TNFa and both T cell and monocyte conditioned media promoted monocyte fusion of some CVID individuals over 5 fold the normal average level, with combinations of cytokines and monokines acting synergistically. The reduction of normal giant cell formation by *anti*-IFNγ antibody and a greater tendency of CVID cells to fuse in immunoglobulin conditioned media suggests that standard IVIg treatment contributes to granuloma formation. CVID and normal giant cells expressed similar levels of phenotypic molecules and had similar phagocytic activity. Monocytes from many CVID patients have an elevated tendency to fuse which may explain the high incidence of granulomatous complications in CVID.

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1. Introduction

Multinucleated cells are observed regularly in the immune response to malignancy [16], and some microbial and parasitic diseases [21]. First reported in patients with tuberculosis over a century ago, multinucleated giant cells are formed by macrophage fusion [34] and represent an end-point in myeloid cell lineage development. Giant cells are a regular feature of the immune response to diseases characterised by pathogen survival in phagocytes, such as leprosy, syphilis and aspergillosis [50]; they are also a component of chronic inflammatory reactions to parasites, including schistosomiasis, *Leishmania* and Filaria [12]. Non-caseating epithelioid cell granulomas occur in about 10% of patients with Hodgkin's lymphoma, and less frequently in other malignant conditions [13], particularly lung, prostate and some skin tumours [33,36].

Granulomatous reactions centred round multinucleate giant cells are a pathological feature of a variety of inflammatory diseases, including sarcoidosis, Crohn's disease and rheumatoid arthritis [6]. Epithelioid or giant cells, which form the centre of granulomatous reactions, are generated in these diseases from macrophages by the action of inflammatory cytokines [29]. Granulomatous reactions are a recognised feature of common variable immunodeficiency (CVID) with about 20% of patients developing unexplained chronic inflammation, often with granuloma formation, involving many different organs, most commonly the spleen, lymph nodes, liver or lungs [2,7]. Although recurrent bacterial infections due to failure of antibody production is the predominant clinical problem in CVID, the circulating T cells and monocytes in many patients show features of persistent activation [27], with evidence that herpes viruses provide a major inflammatory stimulus in some patients [39]. CVID monocytes generally generate defective dendritic cells with deficient major histocompatibility complex (MHC) class II DR expression [48] which induce poor T cell proliferation and abnormal cytokine production [8,18]. Abnormal monocyte responses may contribute to a polarised Th1 immune phenotype in CVID, with exacerbated T cell IL-12 production [15] and increased IL-12 receptor expression [40], promoting a high incidence of granulomatous infiltration of lymphoid and other tissues [54]. In this study, we have investigated the formation of giant cells from CVID monocytes, which could explain the abnormally high rate of granuloma formation in CVID and elucidate the process of inflammatory polarisation in some CVID individuals.

2. Materials and methods

2.1. Patients

Blood samples were obtained from twenty four patients (18 females, 6 males, mean age 49yrs, range 26-75 yrs, Table 1), prior to

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immunoglobulin therapy, attending the Royal Free clinic with a diagnosis of CVID, based on IUIS criteria [17]. Healthy adult volunteers (9 females, 7 males, mean age 40 yrs., range 22–66 yrs.) provided blood samples. Patient and control blood donors were healthy and free from overt signs of infection at the time of donation. All donors gave informed consent and the study had local ethical approval (ref: 04/Q0501/119).

2.2. Cell culture

Peripheral blood was collected in lithium heparin tubes, mixed with an equal volume of X vivo 15 medium (Cambrex Bio Science, Wokingham) and mononuclear cells (PBMCs) separated on Lymphoprep (Axis-Shield ProC As, density 1.077 g/ml) after centrifugation. Plasma supernatants were collected, clarified by centrifugation at 500 ×*g* and used to culture autologous cells. PBMCs were washed twice in medium to reduce platelets and then seeded into 6 well plates (Nunc 152795, Sigma-Aldrich, Poole, Dorset). Routine monitoring of immunologic parameters was performed by the Clinical Immunology Service of the Royal Free Hospital using isotype controls in parallel with each batch of samples analyzed. Peptide stimulation of T cells was performed on fresh PBMCs stimulated with 10 mg/ml CMV or EBV peptides or without peptide in the presence of *anti*-CD28 (10 mg/ml) antibody and brefeldin A (10 mg/ml, Sigma-Aldrich) as previously described [39]. PBMCs were stained with the appropriate HLA pentamer (10 mg/ml) for 15 min at room temperature, washed with PBS containing 0.1% sodium azide, stained for 15 min at room temperature with CD8 FITC and PD-1 APC antibodies (Pharmingen 551347 and 558694), fixed and permeabilized (Fix & Perm Kit, GAS-002, Caltag Medsystems) and stained with a PE-conjugated antibody to granzyme B (Pharmingen, 561142). T cell clonal analysis was performed immediately on a FACSCalibur flow cytometer using a minimum of 50,000 events. Individuals with a >1% total T cell reactivity with CMV tetrameric reagents were designated CMV positive (Table 1).

Adherent monocytes were selected in 2 successive 2 h incubations in 6 well plates, from which non-adherent cells were carefully washed off and adherent cells eluted by incubation with 5 mM EDTA for use in fusion assays. CD14+ monocytes were purified to <98% using an MS column (130-042-201, Miltenyi Biotech, Bisley, Surrey) containing magnetic sheep *anti*-mouse IgG coated beads (Dynal Biotech, 120-000-305) incubated with 1 μg/ml CD14 antibody (BD PharMingen, San Jose, CA, 550376) for 30 min. B lymphocytes (>95% purity) were selected with CD19+ magnetic beads (Miltenyi Biotech,130-052-201) from the non-adherent cell fraction before T lymphocytes (>98% CD3+ purity by flow cytometry) were separated by 2 rounds of negative selection using a mixed antibody (Miltenyi Biotech, 130-091-156). CD14+ monocytes were cultured in X vivo 15 medium with 10% autologous plasma or 10% heat treated (56 °C for 30 min) AB serum (Sera-Lab S-11-J, Haywards Heath, West Sussex) at a density of 5×10^5 per ml at 37 °C in a humidified atmosphere containing 5% CO_2 with a variety of cytokines and mitogens for 7 days. TNFα, IFNγ or IL1α (5 to 50 ng/ml, Peprotech EC, London) were added to some cultures; some culture wells were coated with 10% BSA or type-1collagen (Sigma-Aldrich, C3867) in coating buffer (50 mM Na_2CO_3, pH = 9.5) overnight at 4 °C. Cell viability was monitored by trypan blue exclusion and media including cytokine reagents changed every third day of culture. LPS contamination of media contamination of media (Limulus Amoeba Lysate Kit, Quadratech Ltd., Epsom, Surrey) was below 15 ng/ml.

T cell conditioned media were generated from normal and CVID T lymphocytes incubated overnight in 6 well plates at a density of 1×10^7 cells per ml with 1 μg/ml PHA (nPHA and cPHA, respectively) or in wells coated with 20 μg/ml *anti*-CD3 antibody (nCD3 and cCD3). Antibody coated plates were washed three times with cation-free phosphate buffered saline before use. Normal and CVID monocyte conditioned media was collected from 1×10^7 pooled purified CD14+ normal and patient cells incubated overnight in 6 well plates coated with 10 mg/ml human gamma globulin (Sandoglobulin, CSL Behring, Haywards Heath) (nIVIG and cIVIG, respectively) or 200 nmol/ml phorbol myristate acetate (Sigma-Aldrich) (nPMA and cPMA, respectively). Cell supernatant was clarified by centrifugation for 10 min at

Table 1
Patient immunoglobulin and lymphocyte phenotype.

Patient	Sex	Age	IgG mg/dl	IgA mg/dl	IgM mg/dl	CD3+ $\times 10^9$/l	CD19+ $\times 10^9$/l [a]	Fusion index	CMV status	Clinical presentation
1	F	48	0.1	<0.1	<01	2.04	0.03 (1a)	37.3	+	Bronchiectasis
	F	56	1.5	<0.1	<0.1	1.93	0.03 (1b)	49.2	+	Bronchiectasis, enteropathy
3	M	75	1.6	0.3	0.2	0.84	0.05 (1a)	52.6	+	Granulomatous liver disease
4	M	63	1.0	<0.1	0.7	1.05	0.22 (1b)	16.3	−	
5	M	37	0.1	0.1	0.2	1.98	0.23 (1b)	11.5	−	Bronchiectasis, enteropathy
6	F	61	0.6	<0.1	0.15	1.29	0.24 (1a)	17.7	+	
7	F	55	2.2	0.98	0.41	2.5	0.28 (1a)	18.6	+	Bronchiectasis, enteropathy
8	F	26	3.7	<0.1	0.4	0.94	0.28 (1b)	16.7	−	
9	F	43	2.9	0.1	0.6	1.54	0.29 (1a)	14.5	−	Granulomatous liver disease
10	F	42	4.4	0.1	0.2	1.38	0.29 (1b)	10.8	+	Bronchiectasis
11	F	52	8.8	0.1	0.4	0.72	0.35 (1b)	26.1	nd	Bronchiectasis
12	F	40	2.9	0.42	1.0	1.62	0.36 (1b)	11.3	+	
13	M	48	3.3	<0.1	1.12	0.76	0.38 (1b)	16.2	−	
14	F	70	3.8	0.5	4.6	1.28	0.5 (1b)	12.8	+	
15	F	52	1.1	<0.1	0.2	1.69	1.02 (1a)	48.6	−	Granulomatous disease, bronchiectasis
16	F	59	0.64	<0.1	0.4	2.65	1.11 (1a)	37.4	+	Granulomatous liver disease
17	F	41	0.9	<0.1	<0.1	1.40	nd	34.5	+	Bronchiectasis
18	M	49	0.8	0.8	<0.1	0.78	0.003 (1b)	21.3	−	
19	F	51	0.8	<0.1	<0.1	1.01	0.02 (1b)	24.7	nd	
20	F	56	1.4	<0.1	<0.1	1.75	0.33 (1a)	18.6	nd	
21	F	52	0.9	<0.1	0.4	0.73	0.16 (1b)	23.1	+	Bronchiectasis, enteropathy, granuloma
22	M	22	0.8	<0.1	<0.1	0.55	0.05 (1a)	15.8	+	
23	F	42	1.1	<0.1	<0.1	1.65	nd	41.0	+	Bronchiectasis
24	F	31	2.9	0.2	0.5	1.4	0.35 (1a)	38.9	+	Granulomatous liver disease
R^{2b}			0.023	0.046	0.13	0.022	0.012			
P			0.9	0.83	0.53	0.91	0.96		0.28^	

[a] Freiberg classification.
[b] R^2 correlation to fusion index.
^ *P* value for unpaired *t*-test comparing fusion indices of CMV positive and negative patients.

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