Clin Exp Immunol 1995; 101:89–93

Common variable immunodeficiency (CVID) and MxA-protein expression in blood leucocytes

J. A. RUMP, D. JAKSCHIESS*, U. WALKER, M. SCHLESIER, P. VON WUSSOW* & H. H. PETER
*Abteilung Rheumatologie und Klinische Immunologie, Med. Univ. Klinik, Freiburg, and *Abteilung für Klinische Immunologie, Zentrum Innere Medizin und Dermatologie, Med. Hochschule, Hannover, Germany*

(Accepted for publication 17 March 1995)

SUMMARY

The underlying immunopathogenic mechanism of CVID has been suspected to involve a chronic viral infection or an autoimmune condition. However, formal proof of viral infection is lacking. Measurement of MxA-protein in leucocyte lysates is a sensitive test for evaluating the activation of the host's interferon system. Both viral infections and autoimmune diseases such as systemic lupus erythematosus (SLE) strongly induce MxA-protein in peripheral leucocytes. We therefore examined 15 patients with longlasting hypogammaglobulinaemia for MxA-protein induction *in vivo*: 13 patients suffered from CVID, one from hyper-IgM syndrome, and one patient had chronic B lymphocytic leukaemia associated with immunoglobulin deficiency and chronic papilloma virus infection (condylomata accuminata). Only the latter patient exhibited a strong MxA-protein expression; two CVID patients were borderline positive, and the remaining 12 patients including the hyper-IgM syndrome were MxA-protein-negative. There was no relationship between MxA expression and low CD4/CD8 ratios or increased CD8/CD57$^+$ T cell counts, although both conditions are often observed in CVID as well as in chronic viral infections. When exposed *in vitro* to interferon-alpha (IFN-α), peripheral blood leucocytes of four MxA-negative patients were capable of producing normal amounts of MxA-protein. Taken together, these results argue against a viral or autoimmune pathogenesis of CVID.

Keywords common variable immunodeficiency MxA-protein viral infection interferon-alpha

INTRODUCTION

The pathogenesis of CVID remains poorly understood. The main immunological feature of this disease is an idiopathic hypogammaglobulinaemia of all isotypes, IgM being the least affected. B cell counts and surface isotype distribution are usually normal except for a subgroup of patients with low B cell numbers. Functional and phenotypic disturbances in the T cell compartment do occur, but are subtle and probably non-specific. Attempts to classify CVID are primarily based on clinical features (early or late onset, presence of splenomegaly, autoimmune phenomena, MHC association) [1–6] and certain abnormal laboratory findings such as the ability of peripheral blood lymphocytes (PBL) to synthesize immunoglobulins *in vitro* [7–9], abnormalities in T cell subsets [10–12], impairment of IL-2 production [9,13–16], and involvement of the gut-associated lymphoid tissue [17–19]. Recently a subset of CVID patients was identified with defective CD40 ligand expression [20].

Correspondence: Professor Dr H. H. Peter, Abt. Rheumatologie und Klinische Immunologie, Med. Univ. Klinik, Hugstetterstr. 55, D-79106 Freiburg, Germany.

A persistent lymphotropic viral infection has been suggested as a possible underlying cause of CVID [21,22]. However, diagnosis of viral infections is difficult, since these patients do not produce specific anti-viral antibodies. Previous reports on the isolation of HIV-1 from lymphocytes of three CVID patients have not been confirmed in a larger group by the same authors using polymerase chain reaction (PCR) with HIV-1-specific primers [23]. Similarly, a role for persistent human herpesvirus type 6 (HHV-6) infection has been discussed [23]. On the other hand, Döcke *et al.* described reactivation of cytomegalovirus (CMV) determined by PCR in peripheral lymphocytes of CVID patients [24].

To help clarify this issue we measured interferon-induced MxA-protein expression in PBL lysates of CVID patients as a new surrogate marker for viral infection [25,26]. Interestingly, this sensitive test is regularly positive not only in all viral infections so far tested but also in autoimmune diseases such as systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), and in some patients with rheumatoid arthritis (RA) [32]. The results were compared with lymphocyte surface markers, since a subset of CVID patients with low CD4/CD8 ratio has recently been shown to express increased T

cell activation markers, cytotoxicity and B cell suppressor activity, similar to that seen in viral infections [12,33].

PATIENTS AND METHODS

Patients

Six male and nine female patients with impaired immunglobulin synthesis *in vivo* were tested for spontaneous MxA-protein expression in lysates of their PBL. Their mean age was 44·4 ± 13·8 years, mean time since diagnosis of immunodeficiency was 6·3 ± 5·2 years. Thirteen patients were diagnosed as having CVID (case nos 1–5 and 7–14), one female patient suffered from an autosomal recessive form of the hyper IgM-syndrome (CD40 ligand positive; case no. 15), and one patient (case no. 6) had hypogammaglobulinaemia associated with chronic lymphatic leukaemia (CLL). The latter suffered from papillomavirus-induced severe genital condylomata accuminata and was included in the study as a positive virus carrier control.

Patient nos 1, 7, 11 and 15 suffered from severe respiratory infections, with bronchiectasis and recurrent pseudomonas infection of the lung. Three patients had chronic obstructive lung disease due to intrinsic bronchial asthma (case nos 7, 9 and 11). Five patients (case nos 2, 3, 8 and 13) had recurrent skin involvement such as staphylococcal pyoderma and/or acne vulgaris. Four patients were diagnosed as having chronic atrophic gastritis (case nos 4, 7, 12 and 15).

Five out of the 15 patients had endoscopically and histologically proven lymphoid hyperplasia of the small intestine (case nos 1, 4, 7, 10 and 14).

Ten out of 15 patients were classified according to a modified scheme of Bryant *et al.* [8,9]. These patients were assigned into three groups (A, B and C) according to the ability of their lymphocytes to synthesize IgM and IgG after T cell-independent B cell stimulation with *Staphylococcus aureus* Cowan I (SAC) and IL-2. The three groups were characterized as follows: group A, no IgM and no IgG synthesis; group B, IgM, but no IgG synthesis; group C, IgM and IgG synthesis. Group C patients were further divided into C1 and C2, according to their capacity to synthesize immunoglobulin following pokeweed mitogen (PWM) stimulation [9]. Three patients were assigned to group A (case nos 2, 7 and 11), three to group B (case nos 8, 10 and 13), three to group C1 (case nos 3, 12 and 15), and one patient to group C2 (case no. 4) (Table 1). Four patients remain unclassified so far (case nos 1, 5, 9 and 14), and one patient had CLL (no. 6). Twelve patients received regular intravenous immunoglobulin (IVIG) replacement therapy (Sandoglobulin, Polyglobin N, Endobulin or Intraglobin; 300 mg/kg body weight every 4–6 weeks) keeping their serum IgG levels above 4 g/*l*.

Surface markers

Analysis of lymphocyte surface markers was performed by two-colour flow cytometry using a FACStar. PBL were isolated from venous EDTA blood by Ficoll–Hypaque density gradient centrifugation. Unfractioned mononuclear cells were incubated with directly fluoresceinated MoAbs for 30 min at 4°C. Stained cells were washed twice and then analysed by two-colour flow cytometry in a FACStar (Becton Dickinson, Heidelberg, Germany). T cell markers used were anti-CD3 (Leu-4; Becton Dickinson) and the subset reagents anti-CD4 (Leu-3a; Becton Dickinson) and anti-CD8 (Leu-2a; Becton Dickinson). Activation markers employed were anti-HLA-DR (Becton Dickinson), anti-CD25 (IOT 14; Dianova, Hamburg, Germany) and anti-CD57 (Leu-7; Becton Dickinson). The only B cell marker used was anti-CD20 (Leu-16; Becton Dickinson). The presence of monocytes was excluded by gating for lymphocytes and using anti-CD14 (MY 4; Coulter, Krefeld, Germany) as gate control marker. All antibodies were directly conjugated with either FITC or PE.

Table 1. Clinical data and leucocyte counts in CVID and controls

Patient no.	Age (years)	Sex (F/M)	Duration of CVID (years)	CVID group*	Leucocyte cells/μl	Lymphocyte cells/μl	Lymphocytes, %	Monocytes, %	$CD4^+$ cells/μl	$CD8^+$ cells/μl
1	48	M	4	ND	4400	1535	34·9	5·6	675	737
2	23	M	2	A	9100	1100	12·1	10·1	550	440
3	32	F	5	C1	8700	1690	19·4	5·8	995	625
4	54	F	19	C2	7000	1625	23·2	4·7	650	730
5	31	M	1	ND	2600	1084	41·7	7·0	530	410
6	59	F	3	CLL	10 640	10 180	95·0	2·0	404	607
7	69	F	12	A	4300	765	17·8	10·8	370	250
8	33	F	3	B	7200	1570	21·8	5·0	865	360
9	52	F	12	ND	12 800	896	7·0	3·5	420	260
10	39	M	8	B	3900	1808	48·2	6·5	600	1050
11	48	M	10	A	4600	1075	23·4	10·1	390	580
12	66	F	14	C1	8400	1805	21·5	7·6	630	810
13	30	F	3	B	ND	ND	ND	ND	ND	ND
14	38	M	6	ND	5400	1060	19·7	6·1	490	360
15	44	F	3	C1†	8100	2190	27·0	4·7	635	395
Control range					4000–9400	880–4510	22–48	0.9–9.3	500–900	220–580

* For CVID classification see Patients and Methods.
† Hyper-IgM syndrome.
CLL, Chronic lymphocytic leukaemia; ND, not done.

Table 2. Lymphocyte surface markers and MxA-protein in CVID and controls

Patient no.	$CD4^+$ %	$CD8^+$ %	CD4/CD8 ratio	$CD3^+$ %	$CD20^+$ %	$CD25^+/CD3^+$	$CD25^+/CD20^+$	$CD8^+/CD57^+$	$CD3/HLA\text{-}DR^+$, %	MxA*, U/ml
1	44	48	0·91	93	2·0	2·0	3·0	22	43	0·03 neg
2	50	40	1·25	88	2·0	3·0	0·0	7	6	0·13 (+)
3	59	37	1·59	94	1·0	1·0	0·0	1	1	0·06 neg
4	40	44	0·89	85	ND	1·0	ND	21	9	0·02 neg
5	49	37	1·29	87	6·0	2·0	0·0	9	8	0·08 neg
6	4	6	0·66	9	85·0	0·0	11·0	1	5	0·60 + +
7	48	33	1·48	94	1·0	1·0	0·0	8	5	0·02 neg
8	55	23	2·40	81	9·0	4·0	0·0	11	3	0·19 (+)
9	49	29	1·62	75	7·5	6·0	1·0	7	2	0·04 neg
10	32	54	0·57	85	ND	1·0	ND	34	5	0·03 neg
11	36	54	0·67	92	4·0	2·0	0·0	14	18	0·02 neg
12	35	45	0·77	80	10·0	3·0	0·0	14	13	0·02 neg
13	57	33	1·58	91	6·0	1·0	0·0	6	5	0·02 neg
14	46	34	1·35	85	8·0	2·0	0·0	18	7	0·02 neg
15	29	18	1·60	46	42·0	2·0	5·0	7	3	0·08 neg
Control range	35–72	14–45	1·30–2·30	69–93	1–12	1–7	0·2–2	3–17	1–1	< 0·1

* MxA-protein concentration as determined by whole blood immunoassay.
ND, Not done.

Quantification of MxA-protein in PBL lysates

From each of the 15 patients 5 ml citrated venous blood were collected (Division of Rheumatology and Clinical Immunology, Freiburg) and stored at −80°C until shipment in deep frozen condition to the Division of Clinical Immunology (Hannover, Germany) where the MxA assay was performed on coded samples. PBL (10^6/ml) from four CVID patients (nos 5, 11, 13 and 14) and one healthy control person were incubated for 48 h with 100 U/ml of interferon-alpha (IFN-α). The cell pellet was checked for MxA expression.

Interferon-induced MxA-protein was determined in PBL as described [25]. Briefly, for immunizations and standardizations, recombinant human MxA-protein was expressed in *Escherichia coli* and purified from inclusion bodies by several steps of chromatography. Two MoAbs against non-overlapping epitopes and specific for human MxA-protein were generated to establish a simple two-site immunometric enzyme assay. In addition, a MoAb also reacting with Mx-proteins of other species was identified [25]. Before the assay, whole blood samples were lysed with the nonionic detergent digitonin [25]. The sample was incubated on wells coated with a first MoAb (1304.5.32) followed by a second biotinylated monoclonal (1302.34.16), which, after washing, was revealed by an avidin-alkaline phosphatase system. The detection limit was 5 ng/ml.

RESULTS

The results of CVID classification, leucocyte counts, surface marker analysis and MxA-protein concentrations in cell lysates are summarized in Tables 1 and 2. The only patient who exhibited a strongly positive MxA-protein reaction was patient 6, who suffered from CLL complicated by hypogammaglobulinaemia and papillomavirus-induced chronic condylomata accuminata. This result indicates that the MxA-protein assay can reliably detect a chronic viral infection in a hypogammaglobulinaemic patient. Two other patients (nos 2 and 8) were weakly positive, with MxA-protein values of 0·13 and 0·19 U/ml, respectively (cut-off 0·1 U/ml). The remaining 12 patients were clearly MxA-protein-negative. Interestingly, four of them were tested for MxA-protein expression *in vitro* following a 48-h exposure to 100 U/ml IFN-α. All four patients were perfectly capable of expressing MxA-protein *in vitro* (Table 3).

It has been suggested that those CVID patients with a low CD4/CD8 ratio and high $CD8/CD57^+$ T cell counts might be the most likely candidates for a chronic viral infection [12,13]. Interestingly, in our study the two patients with weakly positive MxA-protein values had normal CD4/CD8 ratios and no increased T cell activation markers, whereas all patients with high $CD8/CD57^+$ T cell counts and low CD4/CD8 ratio (nos 1, 4, 10, 11, 12 and 14) were MxA-protein-negative. These results suggest that chronic viral infections of unknown origin are unlikely to be an underlying cause of low CD4/CD8 counts and T cell activation in CVID. The two patients with borderline MxA-protein concentrations may have suffered from transient viral infections or were unspecifically positive.

No correlation of MxA-protein positivity was observed with other lymphocyte markers or with the classification

Table 3. Expression of MxA-protein in CVID leucocytes upon 48 h exposure to IFN-α (100 U/ml) *in vitro*

Patient	Before, U/ml	After 48 h IFN-α, U/ml
CVID no. 5	0·41	2·90
CVID no. 11	0·05	1·06
CVID no. 13	0·19	1·10
CVID no. 14	0·34	0·90
Control	0·37	2·50

scheme of Bryant *et al.* [8,9]. Also, MxA-protein expression appeared not to be altered by regular IVIG replacement therapy, since patient 6 (CLL plus condylomata accuminata) exhibited high MxA-protein expression while receiving the same IVIG treatment as CVID patients.

DISCUSSION

MxA-protein has been shown to be specifically induced by type I interferons, notably IFN-α [26]. IL-1, tumour necrosis factor-alpha (TNF-α) and other cytokines failed to regulate the Mx-genes [27]. The presence of MxA-protein in nuclei and cytoplasm of PBL is considered to be a sensitive surrogate marker of the activation of the interferon system, and provides evidence in support of an ongoing active viral infection. This concept has been nicely confirmed in a follow-up study of healthy individuals undergoing live virus vaccination against yellow fever [28]. Other viral infections such as herpes simplex virus (HSV), HIV, influenza virus and vesicular stomatitis virus (VSV) have been shown to induce Mx-proteins [29,30]. In contrast, persistent Epstein–Barr virus (EBV) infection in otherwise healthy individuals fails to activate the interferon system sufficiently for MxA-protein production, suggesting that only viral infections which cause a brisk interferon response are capable of inducing MxA. A physiological role of human and mouse Mx-proteins consists of blocking different steps of the influenza virus multiplication cycle [31]. Interestingly, autoimmune diseases such as SLE and MCTD have also been shown to cause MxA-protein expression in PBL [32]. Since both chronic viral infection [12,21–24,33] and autoimmune phenomena [4] have been suggested to be involved in the pathogenesis of CVID, we were interested in the possibility of seeing footprints of a viral infection in this hitherto poorly understood immunologic disorder. However, we found strongly positive MxA-protein expression only in one control patient suffering from CLL, hypogammaglobulinaemia and chronic papillomavirus infection, while the CVID patients were negative. Four of the MxA-protein-negative CVID patients were perfectly capable of producing MxA *in vitro*, ruling out an intrisic MxA-protein defect in CVID lymphocytes. Moreover, a previous study of our group has shown that leucocytes of CVID patients produce normal amounts of IFN-α upon stimulation *in vitro* with HSV and *Corynebacterium parvum* [34].

Our findings are in line with a recent report of Sa'Adu *et al.* [23] who probed lymphocyte DNA from CVID patients by PCR for HIV-1- and HHV-6-specific signals; all samples tested were negative for HIV-1 and only three gave a positive signal for HHV-6. Taken together, our results argue against an underlying chronic viral infection or an SLE-type autoimmune disorder in CVID. They do not rule out, however, a scenario in which CVID may be triggered by a chronic viral infection with a very small virus load insufficient for activating the interferon system.

ACKNOWLEDGMENT

We wish to thank Ms G. Erpenbeck for skilled technical assistence.

REFERENCES

1 WHO Scientific Group on Immunodeficiency. WHO Report: Immunodeficiency. Clin Immunol Immunpathol 1979; **13**:296–302.

2 Asherson GL, Webster ADB, Humphrey JH. Diagnosis and treatment of immunodeficiency disease. Oxford: Blackwell Scientific Publications, 1980.

3 Farrant J, Webster ABD. Different categories of common variable immunodeficiency. In: Eibl *et al.*, eds. Symposium in immunology, Vol. III. Heidelberg: Springer Verlag 1994:91–101.

4 Cunningham-Rundles C. Clinical and immunologic analyses of 103 patients with common variable immunodeficiency. J Clin Immunol 1989; **9**:22–33.

5 Cunningham-Rundles, Lieberman P, Hellman G, Chaganti RS. Non-Hodgkin lymphoma in common variable immunodeficiency. Am J Hematol 1991; **37**:69–74.

6 Primary immunodeficiency diseases. Report of a WHO scientific group. Clin Exp Immunol 1995; **99** (Suppl. 1): 2–24.

7 von Bismarck U, Peest D, Dräger R, Serbin A, Schlesier M, Peter HH. Terminal B-cell maturation and immunoglobulin synthesis *in vitro* in patients with primary and secondary immunodeficiencies. Immun Infekt 1984; **12**:75–87.

8 Bryant A, Calver NC, Toubi E, Webster ADB, Farrant J. Classification of patients with common variable immunodeficiency by B cell secretion of IgM and IgG in response to anti-IgM and Interleukin-2. Clin Immunol Immunopathol 1990; **56**:239–48.

9 Rump JA, Jahreis A, Schlesier M, Dräger R, Melchers I, Peter HH. Possible role of IL2 deficiency for hypogammaglobulinemia in patients with common variable immunodeficiency. Clin Exp Immunol 1992; **89**:204–10.

10 Waldmann TA, Broder S, Krakauer R, MacDermott RP, Durm M, Goldmann C, Meade B. The role of suppressor cells in the pathogenesis of common variable hypogammaglobulinemia and the immunodeficiency associated with myeloma. Fed Proc 1976; **35**:2067–72.

11 Lebranchu Y, Thibault D, Degenne D, Bardos P. Abnormalities in $CD4^+$ T lymphocyte subsets in patients with common variable immunodeficiency. Clin Immunol Immunopathol 1991; **61**:83–92.

12 Jaffe JS, Strober W Sneller MC. Functional abnormalities of CD8 T cells define a unique subset of patients with common variable immunodeficiency. Blood 1993; **82**:192–201.

13 Pastorelli G, Roncarolo MG, Touraine JL, Peronne G, Tovo PA, de Vries JE. Peripheral blood lymphocytes of patients with common variable immunodeficiency (CVI) produce reduced levels of interleukin-4, interleukin-2 and interferon-gamma, but proliferate normally upon activation by mitogens. Clin Exp Immunol 1989; **78**:334–40.

14 Eisenstein EM, Jaffe JS, Strober W. Reduced interleukin-2 (IL-2) production in common variable immunodeficiency is due to a primary abnormality of CD4 T cell differentiation. J Clin Immunol 1993; **13**:247–58.

15 Spickett GP, Farrant J. The role of lymphokines in common variable immunodeficiency. Immunol Today 1989; **10**:192–5.

16 Fischer MB, Hauber I, Vogel E, Wolf HM, Mannhalter JW, Eibl MM. Defective interleukin-2 and interferon-gamma gene expression in response to antigen in a subgroup of patients with common variable immunodeficiency. J Allergy Clin Immunol 1993; **92**:340–52.

17 Herbst EW, Armbruster M, Rump JA, Buscher HP, Peter HH. Intestinal B-cell defect in common variable immunodeficiency. Clin Exp Immunol 1994; **95**:215–21.

18 Nilssen DE, Soderstrom R, Brandtzaeg P *et al.* Isotype distribution of mucosal IgG producing cells in patients with various IgG subclass deficiencies. Clin Exp Immunol 1991; **83**:17–24.

19 Nilssen De, Halstensen TS, Froland SS, Fausa O, Brandtzaeg P. Distribution and phenotypes of duodenal intraepithelial gamma delta T cells in patients with various types of primary B cell deficiency. Clin Immunol Immunopathol 1993; **68**:301–10.

20 Farrington M, Grosmaire LS, Nonoyama S *et al.* CD40 ligand expression is defective in a subset of patients with common variable immunodeficiency. Proc Natl Acad Sci USA 1994; **91**:1099–103.

21 Webster ADB, Datglush AG, Malkowski M *et al.* Isolation of retroviruses from two patients with common variable hypogammaglobulinemia. Lancet 1986; **i**: 581–3.
22 Spickett GP, Millrain M, Beatti R, North M, Griffieths J, Patterson S, Webster ADB. Role of retroviruses in aquired hypogammaglobulinemia. Clin Exp Immunol 1988; **74**:177–81.
23 Sa'Adu A, Thomson BJ, Bountiff L, Webster ADB. Lymphotropic viruses in common variable immunodeficiency: PCR analysis of lymphocyte DNA for HIV-1 and HHV-6. Clin Exp Immunol 1993; **91**:50–53.
24 Döcke WD, Simon HU, Fietze E *et al.* Cytomegalovirus infection and common variable immunodeficiency Lancet 1991; **338**:1597.
25 Towbin H, Schmitz A, Jakschiess D, von Wussow P, Horis-berger MA. A whole blood immunoassay for the interferon-inducible human Mx protein. J Interferon Res 1992; **12**:67–74.
26 Von Wussow P, Jakschiess D, Hochkeppel HK, Fibich C, Penner L, Deicher H. The human intracellular Mx-homologous protein is specifically induced by type I interferons. Eur J Immunol 1990; **20**:2015–9.
27 Simon A, Fah J, Haller O, Staeheli P. Interferon regulated Mx genes are not responsible to IL-1, TNF-α and other cytokines. J Virol 1991; **65**:968–71.
28 Roers A, Hochkeppel HK, Horisberger MA, Hovanessian A, Haller O. MxA gene expression after live virus vaccination: a sensitive marker of type I interferon. J Infect Dis 1994; **169**:807–13.
29 Haller O, Staeheli P. Resistence to influenza virus and vesicular stomatitis virus conferred by expression of human MxA protein. J Virol 1990; **64**:3370–5.
30 von Wussow P, Jakschiess D, Block B *et al.* The interferon-induced Mx homologous protein in people with symptomatic HIV-1 infection. AIDS 1990; **4**:119–24.
31 Pavlovic J, Haller O, Staeheli P. Human and mouse Mx proteins inhibit different steps of the influenza virus multiplication cycle. J Virol 1992; **66**:2564–9.
32 von Wussow P, Jakschiess D, Hochkeppel HK, Horisberger MA, Hartung K, Deicher H. Mx homologous protein in mononuclear cells from patients with systemic lupus erythematosus. Arthritis Rheum 1989; **32**:914–8.
33 Baumert E, Wolff-Vorbeck G, Schlesier M, Peter HH. Immunophenotypical alterations in a subset of patients with common variable immunodeficiency (CVID). Clin Exp Immunol 1992; **90**:25–30.
34 Peter HH, Gronewold P, Kirchner H, Northoff H. Lymphokine production in common varied immunodeficiency, polyclonal hypergammaglobulinemia and granulomatous diseases. In: Oppenheim JJ, Cohen S, eds. Interleukins, lymphokines and cytokines. New York: Academic Press, 1983: 677–85.