Is the mechanism of systemic immune activation in XMRV positive CFS patients similar to that observed in HIV?

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BACKGROUND

Chronic activation of the immune system is a hallmark of progressive HIV infection and is a better predictor of disease outcome than plasma viral load. Brenchley et al[1] showed that circulating microbial products were a cause of HIV-related immune activation, which they called microbial translocation. We have previously shown that Chronic Fatigue Syndrome (CFS) patients suffer from gastrointestinal dysbiosis[2] and from immune dysfunctions[3]. Because of the recent discovery of presence of an infectious retrovirus, XMRV in blood cells of patients with chronic fatigue syndrome by Lombardi et al[4], we wanted to test the hypothesis that the pathophysiology of the systemic immune activation in XMRV positive CFS patients could be similar to the one observed in HIV.

PATIENTS AND METHODS

Sixteen CFS patients fulfilling the Canadian criteria[5] who were found positive for XMRV by co-culture technique[4] (a sensitive cell culture assay for detection of XMRV) were included in the study. Because of the low number of subjects studied we chose to use reference data from a large control population which were collected earlier.

Immunophenotyping was performed in the laboratory of clinical hematology (University Hospital Brussel, Belgium) using standard flow cytometry: T cells (CD3+, CD4+, CD8+, CD4/CD8, CD3+CD16CD56+), B cells (CD19+), NK cells (CD3-CD16CD56+), other (CD2+, CD2+CD25+, CD25+, CD3+HLADR+, CD19+CD5+, CD57+).

Elastase activity was measured in monocytes and lymphocytes using an enzymatic colorimetric assay EnzChek® Elastase Assay kit E-12056 (Molecular Probes, OR, USA).

C4a: an elisa technique was used using Becton Dickinson OptEIA human C4a elisa kit.
IgG3: nephelometry in serum.

Cytokines and sCD14: serum level measurement using Becton Dickinson Cytometric Bead Assay system.

Perforin: mRNA level measured by real-time PCR, using gene expression assay reagents from Applied Biosystems.

Stool IgA: sent to Diagnos-Techs (Seattle, Washington, and analysed in their labs).

STATISICAL ANALYSIS

Parameters	Normal Range	Reference value	Sign. p	Parameters	Normal Range	Reference value	Sign. p
CD3+	982-2508	2238	0.040	CD4+	495-1652	1074	NS
CD57+	60-360	210	0.001	CD8+	376-1119	748	NS
C4a	20-1400	710	<0.001	CD4/CD8	0.9-2.0	1.45	NS
Elastase	0 - 150	75	0.032	CD16CD56	20-113	66.5	NS
Stool IgA	400 - 800	600	<0.001	CD19+	111-401	265	NS
IgG3	>20	40	<0.001	CD3-CD56	64-437	250.5	NS
sCD14	2800-5000	3900	<0.001	CD2+	1158-2680	1919	NS
IL10	0-5	2.5	0.001	CD25+	142-493	317.5	NS
MCP 1	0 – 165	82.5	0.016	HLADR+	28-197	112.5	NS
MIP beta	0-155	77.5	0.031	CD19CD5	12-73	42.5	NS
IL8	0-10	5	0.021	CD5+	1367-2971	2169	NS
				Perforin	250-750	500	NS
				WBC	4000-10000	7000	NS
				IL12	0-5	2.5	NS
				IL 1 beta	0-3	1.5	NS
				IL6	0-5	2.5	NS
				TGF beta	0-290	145	NS
				Alpha TNF	0-6	3	NS

A one sided t-test was used to test the hypothesis that the mean value of the XMRV group is significantly different from the middle of the normal range (representing normal population). A two-sided test was used when abnormal values can occur at both sides of the normal range, otherwise a one- sided test was used. The significance level was set at 0.05. The statistical analysis was performed by Prof. D. Coomans at the department of medical statistics of the faculty of medicine and pharmacy at the Vrije Universiteit Brussel.

RESULTS

The descriptive statistics for the different parameters are presented in a table on this poster.
The number of CD3+ T cells and CD57+ lymphocytes was significantly lower compared to the reference values.
C4a and elastase activity were significantly higher in the XMRV positive CFS population.
Soluble CD14 which codes for LPS in the plasma was significantly higher at $p < 0.001$ compared to the reference population.
XMRV positive CFS patients had significantly higher serum IL-10, MCP-1, MIP-1beta and IL-8 levels.
Serum levels of other cytokines (IL-12, IL-1beta, IL-6, TGFbeta and alpha TNF) were not statistically different compared to the reference values.
Other lymphocyte subsets showed no difference from the reference in the XMRV positive patients.
Stool IgA and IgG3 were statistically lower in the XMRV-positive patients.

CONCLUSION

- The results of this preliminary study in a limited number of subjects show that XMRV positive CFS patients have lower than normal levels of lymphocytes and low numbers of CD57+ lymphocyte subtype as in HIV. The absolute numbers of CD4+ and CD8+ T cells were not statisticaly different from the reference values, but expanding this study to a larger number of patients is necessary to make solid statements in this regard.
- XMRV-positive CFS patients have an activated innate immune system (elastase activity, increased C4a) which could be related to microbial translocation as their sCD14 is significantly higher than expected; sCD14 strongly correlates with plasma LPS[1].
- Low stool IgA (in some of these 16 patients it was undetectable) also points towards a dysfunctional mucosa-associated lymphomal tissue (MALT) in XMRV-positive CFS patients. Furthermore we found that these patients as a group have lower than normal IgG3 serum levels.
- The cytokines IL-8, IL-10, MCP-1 and MIP-1beta are increased and might constitute a biological signature for the viral infection.
- These observations and others (unpublished data on serum levels of LPS in CFS patients) provide evidence for microbial translocation being part of the pathophysiology of XMRV-positive patients.

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