Ann Hematol (2000) 79:110–113

ORIGINAL ARTICLE

H. Bohlen · M. Kessler · M. Sextro · V. Diehl
H. Tesch

Poor clinical outcome of patients with Hodgkin's Disease and elevated interleukin-10 serum levels

Clinical significance of interleukin-10 serum levels for Hodgkin's disease

Received: 8 March 1999 / Accepted: 9 September 1999

Abstract Interleukin (IL)-10 is a pleiotropic cytokine with potent inhibitory effects towards T_H-1 cells. IL-10 inhibits secretion of IL-2 and interferon (IFN)γ by T cells and downregulates major histocompatibility complex antigens. A variety of tumor cells secrete IL-10, which can inhibit growth of tumor-specific cytotoxic T cells. IL-10 expression has also been detected in B-cell lymphomas and Hodgkin's disease (HD), and it has been suggested that the cytokine is involved in the pathogenesis of these tumors. We analyzed levels of IL-10 in pretreatment sera of 64 patients with HD and healthy controls using a sensitive enzyme-linked immunosorbent assay. Patients with biopsy-proven HD were enrolled in trials of the German Hodgkin Study Group (GHSG). Elevated IL-10 levels were detected in the sera of nine patients with HD (14.1%) (range 4.5–225.6 pg/ml with a mean of 61.5 pg/ml). IL-10 was not detectable in a control population of healthy volunteers ($n=90$). Multivariate analyses revealed a significant correlation between elevated IL-10 levels and higher age (over 45 years) but not with any other factors defined by the international prognostic factor score. Patients with elevated IL-10 levels had a significantly lower freedom from treatment failure rate as detected in univariate and multivariate tests. Thus, IL-10 may serve as an independent prognostic factor for HD patients.

H. Bohlen (✉)
Klinik I für Innere Medizin der Universität zu Köln,
J. Stelzmannstr. 9, 50931 Köln, Germany
e-mail: Heribert.Bohlen@Medizin.uni-koeln.de
Tel.: +49-221-4784489
Fax: +49-221-4785912

H. Bohlen · M. Sextro · V. Diehl · H. Tesch
Department I of Internal Medicine, University of Cologne, Germany

M. Kessler
Department of Diagnostic Radiology, University of Cologne, Germany

Key words Cytokine · Hodgkin's disease · IL-10 · Serum · Prognostic marker

Introduction

Interleukin (IL)-10 is a pleiotropic cytokine mainly produced by activated T cells and T_H-2 T-cell clones, activated monocytes/macrophages, stimulated B cells, and mast cells [8]. IL-10 is capable of suppressing cytokine secretion by T_H-1 T cells but also induces proliferation and differentiation of B cells, T cells, and mast cells [9]. In addition, IL-10 suppresses antigen-specific T-cell responses. Hodgkin's disease (HD) is characterized by an abnormal or unbalanced secretion/production of cytokines including IL-10, which supports growth of both neoplastic Hodgkin's and Reed-Sternberg (H-RS) cells and their surrounding reactive bystander cells [5–7, 12, 13]. The correlation between detectable IL-10 serum concentrations and the reduced clinical outcome of a distinct group of HD patients supports a critical role of IL-10 in the immune response between malignant tumor cells and T cells, and may point towards a mainly T_H-2 driven T-cell response.

HD patients show a defect in cellular immune responses, including reduced antigen-dependent proliferation, impaired delayed hypersensitivity reaction, and E-rosette formation, which might be related to aberrant high IL-10 expression [4, 10]. A growth supporting function of IL-10 in HD has not been shown in functional studies, but the inhibitory activities of IL-10 on antigen-presenting cells, such as dendritic cells or T cells may be critical for the pathobiology and clinical outcome of HD. Therefore, the upregulated IL-10 expression in HD-involved tumor tissues may be among the cytokines contributing to the immunological dysfunctions observed in HD, including the lack of cell-mediated cytotoxicity against Epstein–Barr virus (EBV)-related antigens [4].

To further approach the role of IL-10 in the pathogenesis of HD, we measured the serum IL-10 concen-

trations by a sensitive enzyme-linked immunosorbent assay (ELISA) (detection limit = 10 pg/ml) in 73 newly diagnosed, untreated HD patients prior to treatment within multicentric therapeutic trials of the German Hodgkin Study Group (GHSG).

Patients and methods

Patients

All patients were enrolled in the HD4–6 trials of the GHSG. In the HD4 trial, patients in the early stages without clinical risk factors received radiotherapy alone (40 Gy extended field vs 30 Gy extended field and 40 Gy involved field). In the HD5 and the HD6 trials, two chemotherapy regimens were compared – COPP-ABVD and COP-ABV-IMEP. COPP-ABVD consisted of cyclophosphamide, vincristine, procarbacine, prednisone, adriamycin, bleomycin, vinblastine, and dacarbacine; COP-ABV-IMEP consisted of cyclophosphamide, vincristine, prednisone, doxorubicin, bleomycin, vinblastine and ifosphamide, methotrexate, etoposide, and prednisone. In the HD5 trial, patients with intermediate stages (i.e., stages I and II with certain clinical risk factors) were treated with two cycles of chemotherapy followed by 30 Gy extended-field/40 Gy involved-field radiotherapy. In the HD6 trial, patients with advanced stages received four cycles of the alternating chemotherapy regimen.

Patients between 16 years and 65 years of age with biopsy-confirmed HD were eligible. The diagnosis of HD was determined by routine histological examination of tumor tissue, and clinical staging was performed according to the Ann Arbor classification. Exclusion criteria included a positive human immunodeficiency virus (HIV) test, pregnancy, creatinine clearance below 60 ml/min, white blood cell (WBC) count less than 3000/μl, platelet count less than 100,000/μl, serum bilirubin greater than 2 mg/dl, concurrent infections, and severe cardiac, pulmonary or cerebral dysfunction. Each patient provided written informed consent.

Pretreatment evaluation included medical history and physical examination, complete blood count, liver and renal functional tests; erythrocyte sedimentation rate (ESR); chest X ray, abdominal ultrasound, chest, abdominal and pelvic computed tomography (CT), and bone marrow and liver biopsy. Sera of HD patients were collected prior to treatment and stored at –70 °C until use. None of the analyzed patients had any clinical or laboratory evidence of concomitant infectious (active EBV infection) or inflammatory disease or other malignancies. Patient characteristics including B-symptoms are shown in Table 1. Sera of 90 gender- and age-matched healthy blood donors served as the control group.

IL-10 assay

Measurements of human IL-10 were performed using a commercially available sandwich ELISA (Biosource, Calif.) and in accordance with the manufacturer's instructions. Samples were assayed in duplicates and the mean values calculated. The Mann-Whitney U-test was used to compare IL-10 serum levels in different patient subgroups. Correlation between IL-10 values and other clinical or laboratory parameters were assessed by the Spearman's rank correlation or Chi-square coefficient. A *P* value of less than 0.05 was considered significant.

Results and discussion

Overall, IL-10 was detectable in the sera of nine patients (14.1%) with HD but not in any of the healthy controls. In the nine HD patients with positive IL-10 serum levels, the mean ± SEM concentration of IL-10 was 61.5 ± 67.8 pg/ml (range of 4.51–225.6 pg/ml).

We also analyzed whether IL-10 was associated with clinical risk factors as defined by Hasenclever and Diehl [3]. There was no correlation with gender, stage, presence of B-symptoms, histological subtype, bulky disease, extranodal involvement, or more than three lymph-node areas. However, there was a significant correlation between elevated IL-10 levels and higher age [older than 45 years (Table 1)]. Comparison with blood parameters revealed no correlation between elevated IL-10 levels and high ESR, low albumin levels, leucytosis, or lymphocytopenia (Table 2).

Statistical analysis of our patients, all uniformly treated in accordance to the HD6 protocol of the GHSG, indicated that pretreatment IL-10 serum levels predicted a group of HD patients with a strong likelihood to early relapse and reduced rate of freedom from treatment failure (Fig. 1). IL-10-positive HD patients also had a lower overall survival rate; however, this difference did not reach statistical significance yet (Fig. 2). Thus, the increased production of IL-10 in HD can be associated with a poor clinical prognosis and may therefore serve as a prognostic factor.

The clinical and histopathological picture of HD is associated with an unbalanced and/or deregulated cytokine production, locally or systemically, which may contribute to the pathogenesis of HD. HD patients frequently present with elevated serum levels of particularly IL-6 and tumor necrosis factor (TNF), but also soluble adhesion molecules such as endothelial cell/leukocyte adhesion molecule (ELAM) and intercellular adhesion molecule (ICAM). The determination of the serum levels for TNF, sICAM-1 and sELAM in the same patient group showed a correlation with the serum IL-10 levels, while IL-6, IL-8, IL-12, and IL-13 were independent (data not shown).

Here, we demonstrate that IL-10, which inhibits cytokine synthesis and T_H-1-mediated T-cell responses, is

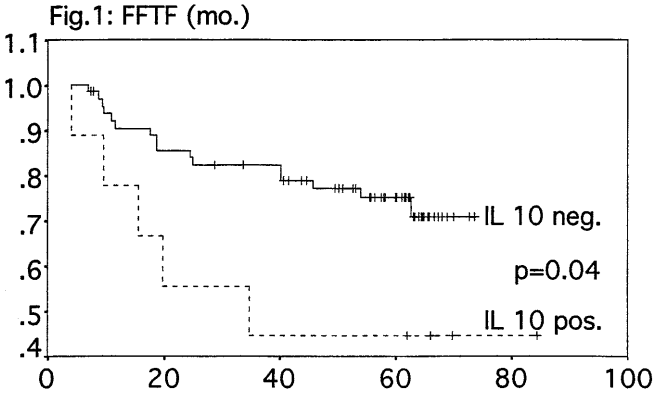

Fig. 1 Freedom from treatment failure estimate of interleukin (IL)-10-positive and IL-10-negative patients. *P* values represent results from univariate comparison between pairs of survival curves using the log-rank test

Table 1 Patient characteristics and correlation of interleukin (IL)-10 serum levels with clinical parameters. *PR*; *PRO*; *CR*; *LP*; *NS*; *MC*; *UC*; *HD*

		Total	IL-10 positive		Pearson Chi-square test (*P*)
		(*n*)	No (*n*)	Yes (*n*)	
Gender	Male	40	36	4	0.505
	Female	33	28	5	
Age	Age <45 years	59	54	5	0.04
	Age >45 years	14	10	4	
Stage	I	9	9	0	0.329
	II	26	24	2	
	III	24	19	5	
	IV	14	12	2	
B-Symptoms	Yes	31	26	5	0.396
	No	42	38	4	
Histology	LP	5	4	1	0.178
	NS	47	44	3	
	MC	20	15	5	
	UC HD	1	1	0	
Bulk	Yes	31	26	5	0.396
	No	42	38	4	
Extranodal involvement	Yes	8	8	0	0.261
	No	65	56	9	
Three or more lymph nodes	Yes	45	38	7	0.288
	No	28	26	2	
Massive splenic involvement	Yes	11	8	3	0.102
	No	62	56	6	
Large mediastinal mass	Yes	13	11	2	0.712
	No	60	53	7	
Score	0–2	62	56	6	0.102
International index	3+	11	8	3	
Therapy outcome	CR	65	57	8	0.707
	PR	5	4	1	
	PRO	3	3	0	
Relapse	Yes	10	7	3	0.067
	No	63	57	6	
Vital status	Alive	66	59	7	0.169
	Deceased	7	5	2	

Table 2 Correlation of interleukin (IL)-10 with blood parameters. *ESR* erythrocyte sedimentation rate

		IL-10 positive		Pearson Chi-square test (*P*)
		No (*n*)	Yes (*n*)	
High ESR	Yes	32	6	0.349
	No	32	3	
Albumin[a]	≥40 g/l	52	6	0.166
	<40 g/l	5	2	
Hemoglobin[a]	≥10.5 g/dl	55	8	0.893
	<10.5 g/dl	8	1	
Leucocytes[a]	<15 g/l	52	7	0.649
	≥15 g/l	10	2	
Lymphocytes[a]	>=8%	53	6	0.158
	<8%	5	2	

[a] Some values were missing

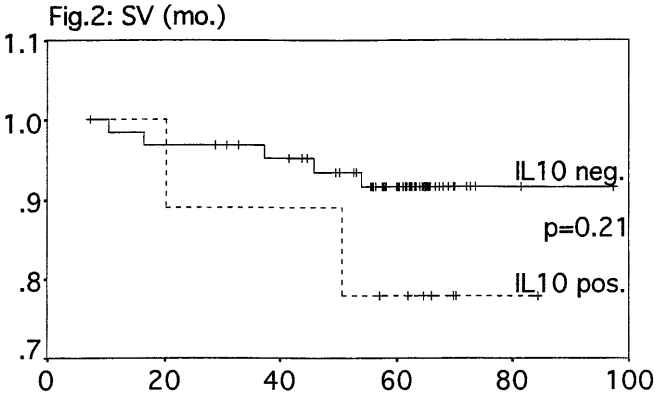

Fig. 2 Overall survival estimate of interleukin (IL)-10-positive and IL-10-negative patients. *P* values represent results from univariate comparison between pairs of survival curves using the log-rank test

detectable in the serum of a fraction of HD patients (14.1%) at diagnosis, whereas it was undetectable in normal blood donors tested. Noteworthy is the correlation between IL-10 serum levels in HD patients and higher age, which is an independent clinical risk factor in patients with advanced HD [3]. Further, the correlation between IL-10 seropositive HD cases and reduced FFTF indicate a prognostic value of serum IL_{10}-levels in HD at diagnosis.

The source of serum IL-10 remains to be determined. It might originate from the malignant H-RS

cells or T cells, surrounding the tumor cells, as demonstrated by in situ hybridization of HD-involved tissue sections [4, 10]. The enhanced production of IL-10 in HD might be related to the deregulated cytokine production and cellular activation by the malignant H-RS cells and could represent a regulative pathway of the host to counteract excessive activity of proinflammatory cytokines, including IL-1, IL-6, and TNF [1, 7, 12, 13]. In line with this assumption, it has been demonstrated that for example TNF is capable of eliciting IL-10 release [11].

Cytokine-mediated cross-regulation is thus regarded as a critical part of the pathogenesis of HD, where IL-10 seems to be involved independently of EBV infection. HD patients with EBV-positive H-RS cells develop an intact humoral immunity against EBV, but fail to develop a cytotoxic immune response against the immunogenic EBV antigens [2]. IL-10 may also provide survival advantage and immune tolerance of EBV-infected H-RS cells by suppression of T-cell and antigen-presenting cell functions in the microenvironment of HD-involved lymphoid tissue. IL-10 might represent a regulatory mechanism to counteract other cytokines and to force a predominant Th2 response. The clinical importance of IL-10 serum levels as a predictive factor for early relapse and reduced clinical outcome needs further detailed analysis in prospective multi-center clinical trials.

Acknowledgements The authors wish to thank all participating centers within the Hodgkin Study Group for their co-operation, especially for providing serum samples and clinical data. Furthermore, special thanks to U. v. Meensel for excellent technical assistance. This study was supported by grants from the Mildred Scheel Stiftung/Deutsche Krebshilfe, the Dörenkamp-Stiftung, the Center for Molecular Medicine Cologue (ZMMK) and by the Deutsche Forschungsgemeinschaft.

References

1. Foss HD, Herbst H, Oelmann E et al. (1993) Lymphotoxin, tumour necrosis factor and interleukin-6 gene transcripts are present in Hodgkin and Reed-Sternberg cells of most Hodgkin's disease cases. Br J Haematol 84:627–635
2. Frisan T, Sjoberg J, Dolcetti R et al. (1995) Local suppression of Epstein-Barr virus (EBV)-specific cytotoxicity in biopsies of EBV-positive Hodgkin's disease. Blood 86:1493–1501
3. Hasenclever D, Diehl V (1998) A prognostic score for advanced Hodgkin's Disease. N Engl J Med 339:1506–1514
4. Herbst H, Foss HD, Samol J et al. (1996) Frequent expression of interleukin-10 by Epstein-Barr virus-harboring tumor cells of Hodgkin's disease. Blood 87:2918–2929
5. Hsu SM, Hsu PL (1994) Autocrine and paracrine functions of cytokines in malignant lymphomas. Biomed Pharmacother 48:433–444
6. Klein S, Jucker M, Diehl V, Tesch H (1992) Production of multiple cytokines by Hodgkin's disease derived cell lines. Hematol Oncol 10:319–329
7. Kretschmer C, Jones DB, Morrison K et al. (1990) Tumor necrosis factor alpha and lymphotoxin production in Hodgkin's disease. Am J Pathol 137:341–351
8. Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR (1993) Interleukin-10. Annu Rev Immunol 11:165–190
9. Mosmann TR (1994) Properties and functions of interleukin-10. Adv Immunol 56:1–26
10. Ohshima K, Suzumiya J, Akamatu M, Takeshita M, Kikuchi M (1995) Human and viral interleukin-10 in Hodgkin's disease, and its influence on CD4+ and CD8+ T lymphocytes. Int J Cancer 62:5–10
11. van der Poll T, Jansen J, Levi M, ten Cate H, ten Cate JW, van Deventer SI (1994) Regulation of interleukin 10 release by tumor necrosis factor in humans and chimpanzees. J Exp Med 180:1985–1988
12. Trumper L, Jung W, Dahl G, Diehl V, Gause A, Pfreundschuh M (1994) Interleukin-7, interleukin-8, soluble TNF receptor, and p53 protein levels are elevated in the serum of patients with Hodgkin's disease. Ann Oncol 1:93–96
13. Xerri L, Birg F, Guigou V, Bouabdallah R, Poizot-Martin I, Hassoun I (1992) In situ expression of the IL-1-alpha and TNF-alpha genes by Reed-Sternberg cells in Hodgkin's disease. Int J Cancer 50:689–693