Pergamon

European Journal of Cancer Vol. 30A, No. 3, pp. 321–324, 1994

0959–8049/94 $6.00+0.00

0959-8049(93)E0034-N

Serum Concentrations of Cytokines in Patients with Hodgkin's Disease

Jean-Yves Blay, Jean-Pierre Farcet, Anne Lavaud, Daniel Radoux and Salem Chouaïb

Serum concentrations of interleukin (IL)-1α, IL-2, IL-4, IL-6 and tumour necrosis factor (TNF) were measured in 24 untreated patients with Hodgkin's disease and in 24 healthy volunteers matched for age and sex. Serum levels of IL-1α were significantly higher in patients with Hodgkin's disease. The number of patients with detectable serum IL-2 or IL-6 levels was significantly higher in patients with Hodgkin's disease as compared to the control group. No difference was observed for TNF. IL-4 was undetectable in all patients. Serum cytokine levels were not significantly different in patients with and without systemic "B" symptoms (weight loss or fever and night sweats) in the different histological subtypes and clinical stages. Serum concentrations of IL-1α, IL-2, IL-6 and TNF were not correlated to the erythrocyte sedimentation rate, fibrinogenaemia or thrombocyte number. These results indicate that subsets of patients with Hodgkin's disease have detectable serum IL-1α, IL-2 and IL-6 levels, but that other mediators are likely to be involved in the associated clinical and biological inflammatory syndrome.
Eur J Cancer, Vol. 30A, No. 3, pp. 321–324, 1994

INTRODUCTION

INTERLEUKIN (IL)-1, IL-6 and tumour necrosis factor (TNF) are proinflammatory cytokines which act as endogenous pyrogens *in vivo* and regulate the synthesis of acute phase proteins by liver cells [1–4]. TNF also mediates the fever observed after IL-2 injection *in vivo* in man [5]. IL-1, IL-6 and TNF are also potent regulators of the growth and differentiation of T and B lymphocytes, monocytes and natural killer (NK) cells as well as hematopoietic progenitors [1–3]. IL-1 and IL-6 exert stimulatory effects on megakaryocyte growth and differentiation both *in vitro* and *in vivo* [6–8]. The production of IL-1, TNF and IL-6 is upregulated by several mediators, including IL-2 [9–12]. In contrast, IL-4 possesses anti-inflammatory activity through the inhibition of TNF, IL-1α and prostaglandin E2 (PGE) production [12–14].

IL-1, TNF and IL-6 act as endocrine hormones in several clinical situations. High levels of circulating TNF have been reported in patients with severe burns [15], septic shock [16], malaria [17] and acute graft versus host (GVH) disease [18]. Similarly, patients with multiple myeloma and renal carcinoma have increased serum IL-6 concentrations which correlate to the prognosis [19, 20].

Hodgkin's disease (HD) is frequently accompanied by systemic symptoms including persistent fever, night sweats and loss of more than 10% of body weight [21]. Increased sedimentation rate, hyperfibrinogenaemia, thrombocytosis, inflammatory anaemia and eosinophilia are also frequently observed in patients with HD. Several lines of evidence suggest that cytokines play a role in the pathogenesis of inflammatory symptoms observed during HD. Hodgkin's tissues from patients with eosinophilia have been reported to express IL-5 mRNA [22]. Fresh Hodgkin's cells express TNF, lymphotoxin, IL-1β and IL-6 mRNA [23–28]; Hodgkin's neoplastic cell lines have been shown to produce detectable levels of TNF, lymphotoxin, IL-1 and IL-6 in the culture supernatants [23, 29, 30]. Yet, no correlation has been found between the presence of systemic symptoms and TNF, lymphotoxin, IL-1 and IL-6 mRNA expression in Hodgkin's tissues [24, 26].

Serum levels of cytokines could be more relevant to inflammatory symptoms than mRNA expression in tumour tissues. We report a study of serum levels of TNF, IL-1α, IL-2, IL-6 and IL-4 in 24 patients with HD and 24 healthy subjects.

PATIENTS AND METHODS

Patients

Serum samples from 24 previously untreated patients with HD (nine females, 15 males, median age 38 years, range 16–74) and from 24 normal healthy volunteers, matched for age and sex, were collected and stored at −20°C. 5 of the 24 patients with HD had systemic "B" symptoms: fever >38°C, night sweats (n = 5) and weight loss of more than 10% (n = 4). Clinical and biological data from patients with Hodgkin's disease are shown in Table 1. Erythrocyte sedimentation rates were determined by the method of Westergreen after 1 h (normal value <20 mm).

Correspondence to J.-Y. Blay at the Centre Léon Bérard, 28 rue Laënnec, 69008 Lyon, France.
S. Chouaïb is at and J.-Y. Blay is also at the UA1156 CNRS, PR1, Institut Gustave Roussy, rue Camille Desmoulins, 94805 Villejuif Cedex; J.-P. Farcet and A. Lavaud are at the Service d'Hématologie, C.H.U. Henri Mondor, 94000 Creteil, France; and D. Radoux is at the Medgenix, Fleurus, Belgium.
Revised 12 Oct. 1993; accepted 11 Nov. 1993.

Assays for cytokines

Cytokines serum levels were measured using ELISA or radioimmunoassay (RIA) kits. Serum levels of TNF were measured using a RIA (IRMA TNF, Medgenix, Fleurus, Belgium; sensitivity 5 pg/ml). IL-1α, IL-2, IL-4, IL-6 serum concentrations were measured using ELISA kits. IL-1α ELISA was purchased from Endogen (Cliniscience, Paris, France; sensitivity 25 pg/ml). IL-2, IL-4 and IL-6 ELISA kits were obtained from Genzyme (TEBU, le Perray-en-Yvelines, France; IT-2 ELISA: sensitivity 0.5 U/ml; IT-4 ELISA: sensitivity 50 pg/ml; IT-6 ELISA: sensitivity 70 pg/ml, Genzyme). Every dosage was performed in duplicate according to the kits procedures and repeated at least twice in separate experiments. Differences of cytokine concentrations between each assay do not exceed 10%. All these assays are highly specific and do not cross-react with other cytokines.

Statistical analysis

Statistical analyses were performed using the non-parametrical Mann–Witney U test and the Fisher's exact test. Analysis of correlations between biological parameters erythrocyte sedimentation rate (ESR), thrombocyte counts, fibrinogenaemia and serum levels of cytokines were performed using Spearman rank's correlation test.

RESULTS

Serum IL-1α, IL-2 and IL-6 concentrations of patients with HD and healthy volunteers are shown in Fig. 1. Patients with HD had significantly higher IL-1α concentrations than healthy volunteers (median 155 versus 43 pg/ml, Mann–Witney U test, $P = 0.002$). 14 (58%) patients with HD had a serum IL-1α level above 150 pg/ml compared to none of the healthy subjects. 17 (71%) HD patients and 9 (38%) controls had detectable serum levels of IL-2 (Fisher's exact test, $P = 0.04$) with median values of 3 and <0.5 U/ml, respectively, in the two groups. 12 (50%) HD patients and 2 (8%) controls had detectable serum levels of IL-6 (Fisher's exact test, $P = 0.003$) with median values of 71 and <70 pg/ml, respectively, in the two groups. Serum TNF was detectable in 6 (25%) HD patients and in 2 (8%) healthy subjects (Fisher's exact test, $P = 0.24$; not shown). IL-4 was undetectable in all patients tested as well as in controls.

IL-1α, IL-2, IL-6 and TNF serum levels were not significantly different (U test, all P values over 0.20) between patients aged under 50 or over 50, in patients with or without fever/night sweats or weight loss, in the different histological subtypes (lymphocyte predominance versus nodular sclerosis versus mixed cellularity) or in clinical stages of the disease (I versus II versus III–IV; Table 1). Using Spearmans rank test, we observed no correlations between serum IL-1α, IL-2, IL-6, TNF levels and thrombocyte counts, ESR or fibrinogenaemia (coefficients between −0.07 and 0.13; P values over 0.50). Furthermore, serum levels of IL-1α, IL-2, IL-6 and TNF were not significantly different (U test, all P values over 0.20) in patients with or without thrombocytosis, hyperfibrinogenaemia or increased ESR.

DISCUSSION

Malignant cells of Hodgkin's disease have been reported to produce several cytokines including IL-1 [26, 29], IL-5 [22], IL-6 [23, 27, 28], TNF and lymphotoxin [26, 30]. So far, the

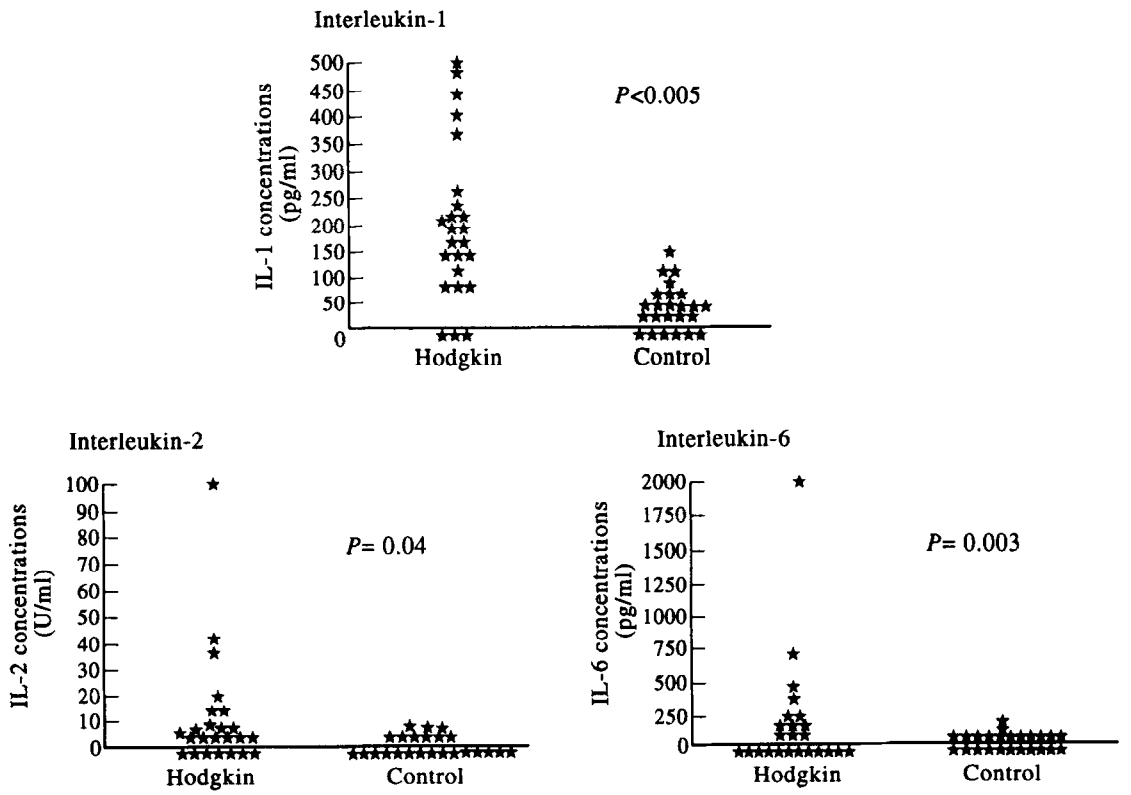

Fig. 1. Concentrations of IL-1α, IL-2 and IL-6 in patients with Hodgkin's disease and in normal subjects. Statistical analyses have been performed with the Mann–Withney U test for IL-1α (median IL-1α levels are 155 and 43 pg/ml, respectively, in HD patients and controls). Serum IL-2 was detectable in 17 (71%) HD patients and 8 (33%) healthy subjects (Fishers exact test, $P = 0.04$). Serum IL-6 was detectable in 12 (50%) HD patients and 2 (8%) healthy subjects (Fishers exact test, $P = 0.003$).

Table 1. Serum cytokines and characteristics of patients with Hodgkin's disease

Patients' characteristics	Number of patients	Serum concentration median (range)			
		IL-1α (pg/ml)	IL-2 (μ/ml)	IL-6 (pg/ml)	TNF (pg/ml)
Age*					
<50	20	190 (<25–500)	2 (<0.5–90)	70 (<70–1900)	<5 (<5–280)
≥50	4	195 (90–316)	4 (2.5–32)	<70 (<70–<70)	<5 (<5–190)
Ann Arbor stage*					
I	7	220 (90–470)	14 (2.5–40)	<70 (<70–1900)	<5 (<5–280)
II	12	210 (<25–500)	<0.5 (<0.5–90)	75 (<70–690)	<5 (<5–250)
III–IV	5	190 (45–420)	5 (3–14)	110 (<70–130	<5 (<5–200)
Histological subtype					
LP	4	155 (75–390)	4 (2.5–32)	<70 (<70–1900)	<5 (<5–190)
NS	17	180 (<25–500)	2 (<0.5–90)	70 (<70–690)	<5 (<5–250)
MC	3	110 (80–260)	4 (<0.5–14)	100 (<70–350)	<5 (<5–280)
Fever and night sweats*					
Absent	19	155 (<25–500)	3 (<0.5–90)	<70 (<70–1900)	<5 (<5–280)
Present	5	250 (120–470)	3 (<0.5–40)	120 (<70–470)	<5 (<5–120)
Weight loss*					
Absent	20	150 (<25–500)	3 (<0.5–90)	<70 (<70–1900)	<5 (<5–280)
Present	4	230 (120–470)	2 (<0.5–40)	70 (<70–470)	<5 (<5–120)
ESR (first hour)†					
<20 mm	10	110 (<25–500)	3 (<0.5–40)	<70 (<70–1900)	<5 (<5–250)
≥20 mm	14	230 (<25–470)	2.5 (<0.5–90)	70 (<70–350)	<5 (<5–280)
Fibrinogen†					
<4 g/l	12	155 (<25–500)	2 (<0.5–90)	<70 (<70–1900)	<5 (<5–250)
≥4 g/l	12	260 (75–470)	3 (<0.5–40)	110 (<70–350)	<5 (<5–280)
Platelets†					
<400 g/l	17	155 (<25–500)	3 (<0.5–90)	70 (<70–1900)	<5 (<5–250)
≥400 g/l	7	210 (75–470)	4 (<0.5–40)	120 (<70–350)	<5 (<5–280)

*Comparisons of cytokine levels between subgroups were performed with the Mann–Withney U test. All P values exceeded 0.2. †Analyses of the correlation between cytokine levels and biological parameters (ESR, thrombocyte counts, fibrinogen) were performed with Spearman's rank correlation test. All P values exceeded 0.50. LP, lymphocyte predominance; NS, nodular sclerosis; MC, mixed cellularity.

production of pro-inflammatory cytokines in Hodgkin's tissues has not been correlated to the clinical presentation of the disease [24–26]. Since cytokines act as endocrine hormones in several clinical situations [15–20], serum concentrations of these mediators could be more relevant to clinical symptoms than mRNA or protein expression in malignant tissues. Our results indicate that patients with HD have significantly higher serum concentrations of IL-1α. IL-2 and IL-6 are also both more frequently detectable in HD patients as compared to normal individuals, whereas serum TNF and IL-4 levels were not significantly different from those of normal subjects. Several studies have demonstrated the presence of IL-1α or IL-1β mRNA as well as IL-1 protein in malignant Hodgkin's cells [24–26, 29].

Production of IL-6 by Hodgkin's cell lines and in fresh Hodgkin's tissue has also been reported [23, 27]. TNF mRNA has been detected in fresh Hodgkin's tissue, but at low levels and in a limited proportion of patients [24, 26]. These results are thus consistent with our data showing high serum levels of IL-1α and IL-6, but not TNF in patients with HD, and suggest that malignant Hodgkin's cells and/or reactive cell populations from Hodgkin's tissue may produce these cytokines in sufficient amounts to be detected in the serum of these patients.

Serum IL-1β was detectable in less than 10% of patients with HD in a recent report [31] whereas in our study 87% of patients with HD had detectable serum IL-1α. IL-1α mRNA was detected in 52% of samples in a recent study [26], whereas IL-1β mRNA was detected in only 26% of the samples in a different report [24]. HD cell lines have been reported to produce IL-1α, but not IL-1β [28]. Taken together, these results suggest a predominant production of IL-1α as compared to IL-1β in patients with HD.

The observation that 50% of our patients with HD have detectable serum IL-6 is in agreement with a previous report [31]. Our data also indicate that serum IL-2 is detectable in a subset of patients with HD. However, in a recent study, IL-2 mRNA was found to be undetectable in HD tissues [27]. A recent report indicates that patients with HD have high serum levels of soluble CD25, the p55 chain of the IL-2 receptor complex [32]. Conceivably, IL-2 detected in patients with HD could actually be produced outside of the tumoral tissues and be trapped by the high levels of circulating CD25 in these patients.

The role of these circulating cytokines in the clinical presentation of HD remains unclear. Our results are in agreement with a previous report, showing the absence of correlations between serum IL-1β, IL-6 or TNF levels and the clinical stage or the histological subtype [31], and further indicate that neither serum IL-1α nor IL-2 correlate to clinical stage or histology.

Although this could be due to the limited number of patients in our series, it is noteworthy that several patients with stage II or III disease had low or undetectable IL-6 or IL-1α serum concentrations whereas patients with stage I disease of the same histological subtype had high serum concentrations of both. This observation, as well as the diversity of cytokines patterns inside the same histological subtype, indicate a possible heterogeneity of Hodgkin's disease in terms of cytokine production.

In our series, all patients with systemic "B" symptoms had detectable serum IL-1α. However, patients with fever or weight loss did not have significantly higher serum IL-1α compared to other patients with HD. Similarly, serum IL-2, IL-6 and TNF levels were comparable in patients with and without fever or weight loss. These results are in agreement with a previous report regarding IL-6 and TNF [31], and further indicate that neither IL-1α nor IL-2 is correlated to the presence of systemic symptoms. Thus, the presence of detectable IL-2, IL-6 or TNF in serum is neither necessary nor sufficient to induce systemic symptoms. However, an involvement of IL-1α cannot be excluded since this cytokine was consistently detected in the serum of patients with systemic symptoms. Although IL-1 and IL-6 induce the production of acute phase protein and stimulate thrombopoiesis both *in vitro* and *in vivo* [1, 3, 6–8], no correlation was found between the presence of an increased ESR, hyperfibrinogenaemia, thrombocytosis and serum concentrations of IL-1α, IL-6 or TNF in our series. This is clearly not due to an insufficient serum concentration of these cytokines since comparable, or even lower, serum cytokine levels have previously been measured in patients with severe clinical conditions or treated with IL-2 [5, 11, 15–17]. Taken together, these results suggest that other pro-inflammatory cytokines or inhibitors of cytokine bioactivity, such as IL-1ra or soluble cytokines receptors, contribute to the pathogenesis of clinical and biological inflammatory syndrome associated with HD [1, 33].

1. Dinarello CA. Interleukin 1 and interleukin 1 antagonism. *Blood* 1991, 77, 1627–1652.
2. Beutler B, Cerami A. Cachectin: a macrophage hormone governing cellular metabolism and cellular response. *Endocr Rev* 1988, **9**, 57–66.
3. Kishimoto T. The biology of interleukin 6. *Blood* 1989, **74**, 1–10.
4. Gauldie J, Richards C, Harnish D, Lansdorp P, Bauman H. Interferon beta 2/BSF2 shares identity with monocyte derived hepatocyte stimulating factor and regulates the major acute phase protein in liver cells. *Proc Natl Acad Sci USA* 1987, **84**, 7251–7255.
5. Mier JW, Vachino G, Van der Meer JWM, *et al.* Induction of circulating tumor necrosis factor alpha as the mechanism of the febrile response to IL-2 in cancer patients. *J Clin Immunol* 1988, **82**, 426–436.
6. Koike K, Nakahata T, Kubo T, *et al.* Interleukin-6 enhances murine megakaryocytopoiesis in serum free culture. *Blood* 1990, 75, 2286–2291.
7. Asano S, Okano A, Ozawa K, *et al. In vivo* effects of recombinant human interleukin 6 in primates: stimulated production of platelets. *Blood* 1990, 75, 1602–1605.
8. Tewari A, Buhles WC, Fletcher-Starnes H Jr. Preliminary report: effects of interleukin 1 on platelets counts. *Lancet* 1990, **ii**, 712–714.
9. Nedwin GE, Svedersky LP, Bringman TS, Palladino MA, Goeddel DV. Effect of IL-2, interferon gamma and mitogens on the production of TNFα and TNFβ. *J Immunol* 1985, **135**, 2492–2497.
10. Numerof RP, Aronson FR, Mier JW. IL-2 stimulates the production of IL-1α and IL-1 beta by human peripheral blood mononuclear cells. *J Immunol* 1988, **141**, 4250–4257.
11. Blay J-Y, Favrot MC, Negrier S, *et al.* Correlation between clinical response to IL-2 therapy and sustained production of tumor necrosis factor. *Cancer Res* 1990, **50**, 2371–2374.
12. Blay J-Y, Branellec D, Robinet E, Gay F, Chouaib S. Involvement of cAMP in the inhibitory effect of IL-4 on IL-2 induced LAK generation. *J Clin Invest* 1990, **85**, 1909–1913.
13. Hart PR, Vitti GF, Burgess DR, Whitty GA, Picolli DS, Hamilton JA. Potential antiinflammatory effect of IL4: suppression of human TNFα, interleukin 1 and prostaglandin E2. *Proc Natl Acad Sci USA* 1990, **86**, 3803–3807.
14. Te Velde A, Huijbens RJF, Heije K, De Vries J, Figdor CG. Interleukin 4 inhibits secretion of IL-1β, TNF and IL-6 by human monocytes. *Blood* 1990, **76**, 1392–1397.
15. Nijstein MWN, DeGroot ER, Ten Duis JH, Hack CE, Aarden LA. Serum levels of interleukin 6 and acute phase responses. *Lancet* 1987, **ii**, 921.
16. Waage A, Halstensen A, Espevik T. Association between tumor necrosis factor in serum and fatal outcome in patients with meningococcal disease. *Lancet* 1988, **i**, 355–359.
17. Grau G, Taylor TE, Molyneux ME, *et al.* Tumor necrosis factor and disease severity in children with falciparum malaria. *N Engl J Med* 1989, **320**, 1586–1591.
18. Holler E, Kolb HJ, Liesenfeld S, *et al.* Increase of TNF serum levels in major transplant related complications of human bone marrow transplantation—association with acute graft versus host disease and activation of host macrophages. In Freund M, Link H, Welte K, eds. *Cytokines in Hematopoiesis, Oncology and AIDS*. Heidelberg, Springer Verlag, 1990, 653–660.
19. Bataille R, Jourdan M, Zhang XG, Klein B. Serum levels of interleukin 6, a potent myeloma growth factor, as a reflect of disease severity in plasma cell dyscrasias. *J Clin Invest* 1988, **84**, 2008–2010.
20. Blay J-Y, Negrier S, Combaret V, *et al.* Serum level of interleukin 6 as a prognosis factor in metastatic renal cell carinoma. *Cancer Res* 1992, **52**, 3317–3323.
21. Hellman S, Jaffé E, De Vita VT Jr. Hodgkin's disease and non Hodgkin's lymphomas. In De Vita Jr V, Hellman S, Rosenberg SA, eds. *Cancer Principles and Practice of Oncology*. New York, Lippincott, 1989, 1694–1740.
22. Samoszuk M, Nasen L. Detection of IL-5 messenger RNA in Reed-Sternberg cells of Hodgkin's patients with eosinophilia. *Blood* 1990, 75, 13–16.
23. Jucker M, Abts H, Li R, *et al.* Expression of Il-6 and IL-6 receptor in Hodgkin's disease. *Blood* 1991, 77, 2413–2418.
24. Sappino AP, Seelentag W, Pelte MF, Alberto P, Vassali P. Tumor necrosis factor/cachectin and lymphotoxin gene expression in lymph nodes from lymphoma patients. *Blood* 1990, 75, 958–962.
25. Ree HJ, Crowley JP, Dinarello CA. Anti interleukin 1 reactive cells in Hodgkin's disease. *Cancer* 1987, **59**, 1717–1723.
26. Xerri L, Birg F, Guigou V, Bouabdallah R, Poizot-Martin I, Hassoun J. *In situ* expression of the IL-1 alpha and TNF-alpha genes by Reed-Sternberg cells in Hodgkin's disease. *Int J Cancer* 1992, **50**, 689–693.
27. Merz H, Fliedner A, Orscheschek K, *et al.* Cytokine expression in T-cell lymphomas and Hodgkin's disease. Its possible implication in autocrine or paracrine production as a potential basis for neoplastic growth. *Am J Pathol* 1991, **139**, 1173–1180.
28. Tesch A, Feller AC, Jücker M, Klein S, Merz H, Dielh V. Activation of cytokines in Hodgkin's disease. *Ann Oncol* 1992, **3** (Suppl. 4), S13–S16.
29. Hsu SM, Zhao X. Expression of interleukin 1 in H-RS cells and neoplastic cells from true histiocytic lymphoma. *Am J Pathol* 1986, 125, 221–225.
20. Hsu PL, Hsu SM. Production of tumor necrosis factor alpha and lymphotoxin by cells of Hodgkin's neoplastic cell lines HDLM-1 and KM-H2. *Am J Pathol* 1989, **135**, 735–745.
31. Gause A, Keymis S, Scholz R, *et al.* Increased levels of circulating cytokines in patients with untreated Hodgkin's disease. *Lymphokine Cytokine Res* 1992, **11**, 109–113.
32. Gause A, Jung W, Schmits R, *et al.* Soluble CD8, CD25 and CD30 antigens as prognostic markers in patients with untreated Hodgkin's lymphoma. *Ann Oncol* 1992, **3** (suppl. 4), S49–S52.
33. Van Zee KJ, Kohno T, Fischer E, Rock CS, Moldawer LL, Lowry SF. Tumor necrosis factor soluble receptors circulate during experimental and clinical inflammation and can protect against excessive tumor necrosis factor alpha *in vitro* and *in vivo*. *Proc Natl Acad Sci USA* 1992, **89**, 4845–4849.

Acknowledgements—This work was supported by grants from La Ligue Nationale Contre Le Cancer, the Institut National pour la Santé et la Recherche Médicale (CRE3458) and Le Comité Départemental du Rhone de la Ligue Contre la Cancer.