original paper
Haematologica 1994; 79:406-412

SERUM LEVELS OF CYTOKINES AND SOLUBLE ANTIGENS IN POLY-TRANSFUSED PATIENTS WITH β-THALASSEMIA MAJOR: RELATIONSHIP TO IMMUNE STATUS

Gina Lombardi, Rosella Matera, Maria Marta Minervini*, Nicola Cascavilla*, Palmina D'Arcangelo°, Mario Carotenuto*, Giuseppe Di Giorgio, Pellegrino Musto**

*Transfusional Service, *Division of Hematology and °Central Laboratory, IRCCS "Casa Sollievo della Sofferenza" Hospital, San Giovanni Rotondo, Italy*

ABSTRACT

Background. **A series of immunological abnormalities has been described in patients with β-thalassemia. The aim of this study was to investigate whether the measurement of serum levels of selected cytokines and soluble molecules (deriving from cell membrane antigens) involved in the immune response could be useful for a better definition of such alterations.**

Patients and Methods. **Serum levels of interleukin-2 (IL-2), IL-6, tumor necrosis factor (TNF), soluble (s) CD4, sCD8, sCD23 and sCD25 were measured using immunoenzymatic assays in 45 transfusion-dependent patients affected by β-thalassemia major and correlated to *conventional* immunological indexes, such as peripheral lymphocyte subpopulations and circulating immunoglobulins.**

Results. **Patients with β-thalassemia major showed increased TNF, sCD8, sCD23 and sCD25 and lower sCD4 values compared to normal controls. IL-2 and IL-6 were found to be undetectable or within the normal range in all patients. Splenectomized patients presented lower levels of sCD8 and sCD23 than those observed in unsplenectomized ones. A series of correlations involving TNF, sCD8, sCD23, sCD25, serum immunoglobulins and some lymphocyte subpopulations was observed. In addition, serum markers of *immune activation* (TNF, sCD23, sCD25) correlated directly with the annual blood transfusion requirement. Despite this series of immunological anomalies, no patient had a history of repeated infectious episodes.**

Conclusions. **Polytransfused β-thalassemic patients are characterized by a partial functional immunodeficiency determined by increased activity of CD8+ suppressor/cytotoxic lymphocytes and possibly reduced activity of the CD4+ helper/inducer subset. B-lymphocytes also appear highly activated. The allo-antigenic stimulation of transfusions seems to play a major role in the determination of these defects; however, this *functional* immunological imbalance does not seem to have any clinical relevance.**

Key words: β-thalassemia, IL-2, IL-6, TNF, sCD4, sCD8, sCD25, sCD23, cytokines, soluble molecules

Several immunological defects have been found in patients affected by β-thalassemia.[1] They are mainly represented by impaired activity of monocytes and neutrophils,[2,3] increased synthesis of polyclonal immunoglobulins,[4,5] defective activity of the complement alternative pathway,[6] and functional or numerical alterations of different peripheral blood lymphocyte subpopulations.[7-12] In general, such abnormalities are thought to be a secondary effect of transfusions and, to a lesser degree, of iron overload or splenectomy.[7,10,13,14] However, it is still not clear if these immunological modifications reflect a real, acquired status of immunodeficiency and, above all, if they have some clinical relevance.

Correspondence: Pellegrino Musto, MD, Division of Hematology, IRCCS "Casa Sollievo della Sofferenza" Hospital, 71013 S.Giovanni Rotondo, Italy. Tel. international +39.882.410539. Fax. international +39.882.411705.
Received January 14, 1994; accepted June 30, 1994.

A novel approach to the problem is now provided by the measurement of serum levels of those cytokines and soluble antigens which exert relevant activities in modulating the different phases of the immune response. For example, recent evidence suggests that interleukin-8 (IL-8),[15] tumor necrosis factor (TNF)[16] and a soluble receptor of IL-2 (sCD25)[17] may have biological and clinical importance in thalassemic patients.

The aim of the present study was to determine the serum levels of a series of cytokines and soluble(s) molecules (deriving from cell surface antigens), and to correlate these findings to the immune status in patients affected by β-thalassemia major. Cytokines (IL-2, IL-6 and TNF) and soluble molecules (sCD4, sCD8, sCD23, and sCD25) were selected on the basis of their synergic participation in the regulation of immune response and their capacity to provide information about the functional status of various lymphocyte subsets. In particular, within the context of a large series of pleiotropic activities, IL-2 plays a major role in the mechanisms of activation and proliferation of T, NK and LAK cells;[18] IL-6 is a potent inducer of the proliferation and production of immunoglobulins in B-lymphocytes,[19] and TNF is largely involved in the phenomena of *inflammation*.[20] On the other hand, the soluble molecules sCD4,[21] sCD8,[22] sCD23[23] and sCD25[24] represent circulating fragments of cell membrane structures that interact with external antigens (CD4 and CD8) or exert receptorial activity (CD23, CD25).[25] They are mainly released by specific lymphocyte subsets, such as helper/inducer, suppressor/cytotoxic, and activated cells, respectively. Serum levels of these soluble molecules are strictly dependent on the absolute number of cells carrying the antigen on their surface, as well as on the functional status of such cells; thus, they offer an indirect marker of such activation.

Patients and Methods

Forty-five patients with β-thalassemia major entered the study (22 males and 23 females, mean age 15 years, range 1-40 years). Five patients had previously undergone splenectomy, five to ten years before the present evaluation. At the time of the study all patients maintained their level of hemoglobin in the range of 9 to 11 g/dL by means of packed red cell transfusions and received regular subcutaneous iron-chelating therapy with desferrioxamine. The number of transfusions received by individual patients ranged from 50 to 330. Along with a careful medical history for previous infectious diseases, hemato-biochemical work-up and broad serological screening for viral infections, a *conventional* immunological profile was performed in all thalassemic subjects. This included cytofluorymetric evaluation of B, T (helper/inducer, suppressor/cytotoxic, γ/δ) and natural killer circulating lymphocyte subpopulations (using anti-CD3, CD4, CD8, CD16, CD20, HLA-Dr and TCRδ1/δTCS1 monoclonal antibodies), and measurement of circulating IgG, IgA and IgM. In selected cases, membrane double fluorescence studies were carried out. Serum levels of cytokines (IL-2, IL-6 and TNF) and soluble molecules (sCD4, sCD8, sCD23 and sCD25) were measured on frozen (–20°) samples drawn at least 18 days after the last transfusion, using the following commercially available immunoenzymatic assays: CELLFREE (sCD4, sCD8 and sCD23) and BIOKINE (IL-6) Test Kits, T-Cell Diagnostics, Cambridge, United Kingdom; Medical Systems, Genova, Italy; SOLUBLE IL-2 RECEPTOR (sCD25) ELISA and IL-2 EIA, Technogenetics, Milano, Italy; TNF EASIA, Medgenix Diagnostics, Brussels, Belgium. No patient had recent or active infection at the time of the determination. Twenty-five healthy blood donors were employed as normal controls. Student's t-test, chi-square test and Pearson's coefficient were used, as appropriate, for statistical analysis.

Results

No patient was found to be HIV, HTLV-1 or HTLV-2 positive. However, 80% of them were HCV positive (RIBA test) and 100% revealed IgG antibodies directed against CMV and EBV. All patients were positive for at least one HBV marker. Direct and/or indirect Coombs' test was positive in 8 patients. Clinical effects of iron

overload were evident in 17 patients (8 diabetes mellitus, 3 hypothyroidism, 6 myocardiopathy), but no one referred an increased incidence of infectious episodes.

Results of the conventional immunological analysis are listed in Table 1. High levels of IgG and IgA were also observed in splenectomized patients (data not shown).

Figures 1-3 illustrate the serum levels of TNF and soluble molecules tested. β-thalassemic patients demonstrated significantly higher mean values of TNF, sCD8, sCD23 and sCD25 and lower levels of sCD4 than those observed in the controls.

An inverse correlation was found between sCD8 values and the absolute number of total, $CD8^+$, $CD3^+$, $CD4^+$ lymphocytes and the CD4/CD8 ratio (Table 2). TNF levels were directly correlated to sCD25, while an inverse

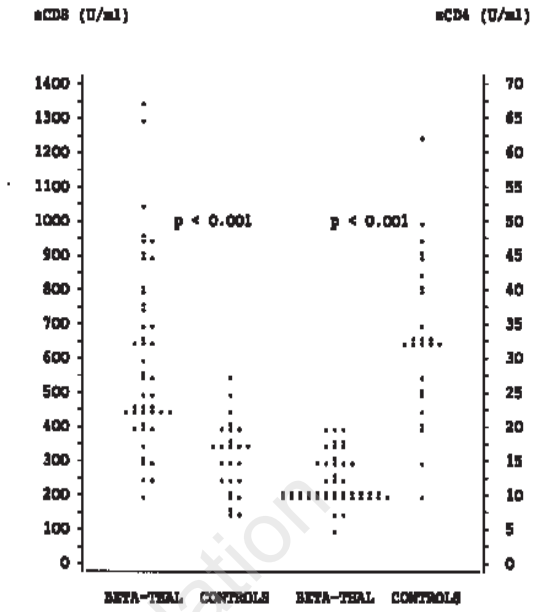

Figure 1. Serum levels of sCD8 and sCD4 in polytransfused β-thalassemic patients and in normal controls.

Table 1. "Conventional" immunological profile in thalassemic patients and in controls. Values are expressed as mean± standard deviation of absolute number/mL (a.n.) or percentage (%) for lymphocyte subsets, and as mg/dL for immunoglobulins. *p < 0.05; **p < 0.01; ***p < 0.001.

	β-thal	controls
Lymphocytes (a.n.)	2712±1346	2300±1115
CD3 (%)	66.9±10.1	62.5±9.8
CD3 (a.n.)*	1764±802	1426±911
CD4 (%)	36.5±6.6	35.5±6.8
CD4 (a.n.)	948±923	816±521
CD8 (%)	25.9±7.7	22.5±5.8
CD8 (a.n.)**	704±380	517±286
CD20 (%)	17.4±4.7	22.5±6.9
CD20 (a.n.)	486±374	518±402
CD16 (%)	8.9±5.1	8.5±4.8
CD16 (a.n.)*	245±190	184±122
HLA-Dr (%)	21.8±5.4	27.0±8.1
HLA-Dr (a.n.)	631±513	621±597
TCRDelta1 (%)	4.7±4.0	4.3±3.8
TCRDelta1 (a.n.)	123±99	115±86
DeltaTCS1 (%)	1.9±1.4	1.4±0.9
DeltaTCS1 (a.n.)	46±36	43±33
CD4/CD8 ratio	1.6±0.8	1.6±0.6
IgG***	1544±445	1330±220
IgA	189±113	227±103
IgM*	212±97	165±52

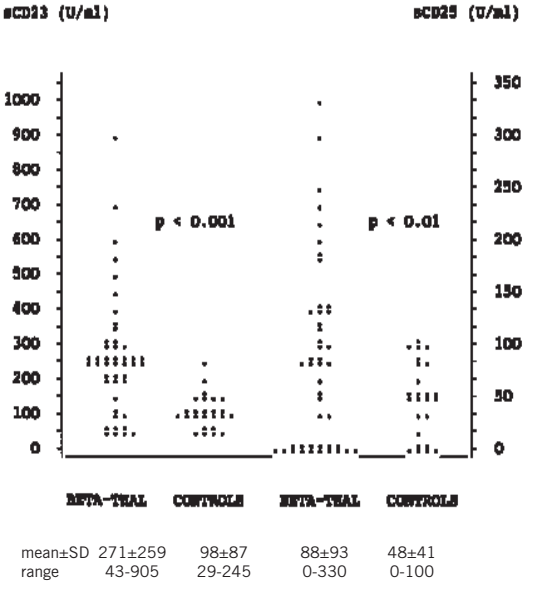

Figure 2. Serum levels of sCD23 and sCD25 in polytransfused β-thalassemic patients and in normal controls.

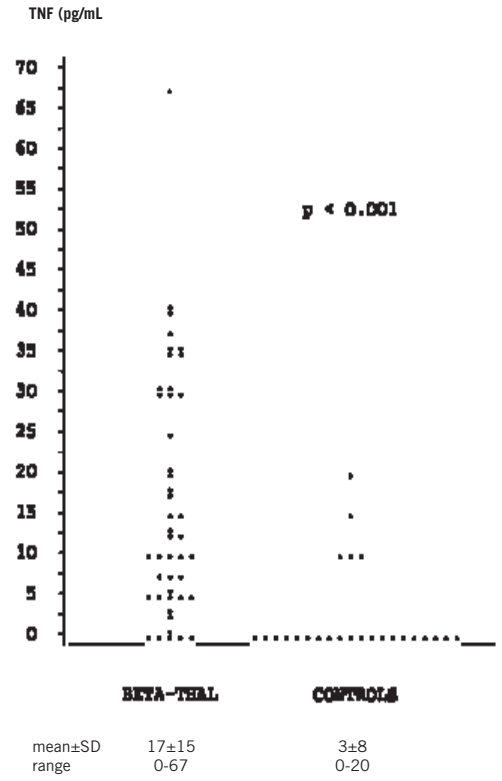

Figure 3. Serum levels of TNF in polytransfused β-thalassemic patients and in normal controls.

between serum levels of sCD23 and IgA (Table 2). Lower values of sCD23 and sCD8 were detected in splenectomized patients than in unsplenectomized ones, but no substantial differences emerged with regard to TNF, sCD4 or sCD25 levels in these two groups (Table 3).

No statistical differences were found between serum levels of IL-2 and IL-6 in β-thalassemic patients and in normal controls (Table 4), although most of patients showed undetectable levels of these two cytokines.

Finally, no correlation emerged between TNF or IL-6 and some acute phase proteins, such as C-reactive protein or fibrinogen (data not shown).

Discussion

In our study, patients with β-thalassemia major showed a significant increase in mean

Table 2. Correlations observed in patients with β-thalassemia major between cytokines or soluble molecules and lymphocyte subpopulations, serum levels of immunoglobulins and annual transfusional requirements (expressed as mL/kg/year). In all remaining possible combinations p was not significant.

sCD8/total lymphocytes	r - 0.96	p < 0.000001
sCD8/CD3+ lymphocytes	r - 0.92	p < 0.000001
sCD8/CD4-CD8 ratio	r - 0.76	p < 0.000001
sCD8/CD8+ lymphocytes	r - 0.68	p < 0.000001
sCD23/IgA	r - 0.69	p < 0.001
sCD8/CD4+ lymphocytes	r - 0.45	p < 0.008
sCD25/transfusions	r 0.48	p < 0.01
TNF/CD4-CD8 ratio	r - 0.41	p < 0.02
TNF/sCD25	r 0.40	p < 0.02
TNF/transfusions	r 0.41	p < 0.02
sCD23/transfusions	r 0.42	p < 0.02

sCD8, sCD23, sCD25 and TNF serum levels with respect to normal values. By contrast, a reduction of circulating sCD4 was also found. The changes in sCD4 and sCD8 did not correspond to substantial alterations of the mean values of CD4/CD8 ratio.

CD4 and CD8 molecules are cell membrane glycoproteins, mainly expressed on lymphocytes, with helper/inducer and suppressor/cytotoxic activity, respectively. This activity plays an important role within the complex mechanisms of cell-to- antigen and cell-to-cell contact and adhesion, as well as in the transduction of antigenic recognition signals.[26-28] We speculate that the low levels of sCD4 we found in thalassemic subjects might reflect a status of functional quiescence of the $CD4^+$ helper/inducer lymphocytes, a subpopulation which was even numeri-

Table 3. Comparison of serum levels of TNF and soluble molecules in splenectomized and unsplenectomized β-thalassemic patients. *p < 0.001; **p < 0.01. For other comparisons: p not significant.

	splenectomized (N.5)	*unsplenectomized (N.40)*
TNF (pg/mL)	28±32	16±14
sCD25 (U/mL)	108±89	85±99
sCD8 (U/mL)*	260±151	690±246
sCD4 (U/mL)	10±2	12±4
sCD23 (U/mL)**	61±49	288±263

Table 4. Serum levels of IL-2 and IL-6 in β-thalassemic patients and normal controls. No significant difference was found.

	β-thal (N.45)	controls (N.25)
IL-2 (U/mL)		
mean+SD	0.06±0.1	0.1±0.3
range	0-0.6	0-1.2
IL-6 (U/mL)		
mean+SD	7±11.2	13.1±15.6
range	0-42	0-83

cally increased in these patients. The low levels of serum IL-2 we observed in most of patients are also in line with such an interpretation. High serum levels of sCD8, as an expression of functional activation of $CD8^+$ suppressor/cytotoxic lymphocytes, were inversely correlated to the CD4/CD8 ratio and to the absolute number of $CD4^+$ and $CD3^+$ lymphocytes. However, an inverse correlation was also found between sCD8 values and the number of circulating $CD8^+$ lymphocytes. Although such an unexpected finding has been recently described in patients with Hodgkin's disease,[29] this observation remains quite surprising since sCD8 serum levels, as well as all soluble circulating antigenic molecules, depend mainly on the number of activated cells which carries the CD8 antigen. As a possible explanation for this phenomenon, we hypothesize a recirculation of $CD8^+$ lymphocytes to areas other than the peripheral blood. In fact, our splenectomized patients showed normal values of sCD8, thus suggesting that the spleen might contain a large number of CD8+ lymphocytes producing sCD8 in β-thalassemic patients. However, other possibilities such as higher expression and more rapid turnover of the membrane CD8 molecule in patients with a low number of strongly activated circulating $CD8^+$ lymphocytes, the fact that other cells may contribute to the serum level of sCD8, or even a defect of clearance cannot be completely ruled out. The activation of $CD8^+$ lymphocytes in β-thalassemic patients (confirmed by membrane double-fluorescence studies performed in selected cases) was further stressed by the high levels of sCD25 we found in these patients. It has been suggested that sCD25, a truncated portion of the cellular receptor of IL-2, could interfere with its circulating ligand, thus reducing the biological activity of IL-2.[30] The high levels of serum TNF, which in our patients were directly related to those of sCD25 and showed an inverse correlation with CD4/CD8 ratio, might further contribute to this picture of immunological imbalance; TNF increases the production of sCD25 in activated T-cells,[31] and a direct relationship between these two molecules has also been reported in acute leukemias with a monoblastic component.[32] Both TNF and sCD25 have been previously found to be increased in the sera of thalassemic patients. In particular, high levels of TNF correlated with the non-DQw1 HLA phenotype, the presence of severe hepatic fibrosis, and the appearance of immunological complications after allogeneic bone marrow transplantation in these patients.[16] On the other hand, high sCD25 levels were found to be produced by activated circulating T-lymphocytes, and to be correlated to the number of annual transfusions received, thus suggesting a relationship with chronic immunological stimulation due to transfusional support.[17]

sCD23[33] is a truncated form of the membrane antigen CD23, which represents the low affinity receptor for IgE and is normally expressed on various hemopoietic cells. In particular, CD23 is expressed in a limited percentage of normal circulating activated B-lymphocytes, where it probably also acts as a B-cell growth factor receptor. The high sCD23 levels we observed confirm the strong activation of B- lymphocytes (even numerically reduced) that occurs in polytransfused thalassemic patients. As a likely consequence, we also found high levels of IgG and IgM, but not of IgA, which were instead inversely correlated to values of sCD23. Since splenectomized patients showed normal sCD23 values, it is possible that this increase in circulating immunoglobulins may be sustained by B-cell populations producing IgG and IgM with a splenic homing. However, this does not explain the reason for the high levels of IgG and IgA

observed in splenectomized patients in the present and in other studies.[4,5] In this setting, it might be interesting to study possible relationships between sCD23 and production of IgE in thalassemic patients, in whom this class of immunoglobulins is often increased and may have a possible role in the incidence of transfusional reactions.[34]

The reason for the low or normal serum levels of IL-6, a potent inducer of B-lymphocyte differentiation and of their capacity to synthesize immunoglobulins, despite strong stimulation of the B-immune system such as that described in our patients with β-thalassemia is not clear; some possibilities have to be considered:

i) reduced activity of $CD4^+$ lymphocyte subsets, which indirectly induce the production of IL-6 via IL-2;

ii) the technical limits of the immunoenzymatic assay employed;

iii) the fact that IL-6 may be active locally at very low concentrations, without the need to reach high systemic levels.

Finally, serum markers of *immune activation* (TNF, sCD23, sCD25) showed a clear correlation with transfusion requirement, thus suggesting a fundamental role for chronic transfusion-related alloantigenic stimulation in determining the immunological abnormalities observed in these patients.[35] The fact that a group of less heavily transfused patients with β-thalassemia intermedia showed normal levels of the cytokines and soluble molecules tested in the present study (personal unpublished data) is in agreement with this hypothesis.

Polytransfused subjects with β-thalassemia major are at risk for viral infections.[36,37] In addition, they may show a complex pattern of immunological abnormalities characterized by activation of both the suppressor/cytotoxic $CD8^+$ subset and B-lymphocytes, associated with a possible reduction in the activity of the $CD4^+$ helper/inducer population. These modifications, which are likely due to transfusional overstimulation of the immune system, probably lead to a *functional* status of partial immunodeficiency, which, nevertheless seems to have no substantial clinical relevance. However, evaluation of the serum levels of selected cytokines and soluble molecules may be a useful tool in improving our knowledge about this functional immunological defects in β-thalassemia.

References

1. Neri A, Callea V, D'Ascola DG, Martino B. Immune status in thalassemia major. Haematologica 1986; 71:511-6.
2. Skoutelis AT, Lianov E, Papavassiliou T, Karameron A, Politi C, Bassaris HP. Defective phagocytic and bactericidal function of polymorphonuclear leucocytes in patients with beta-thalassemia major. J Infect 1984; 8:118-22.
3. Ballart IJ, Estevez ME, Sen L, et al. Progressive dysfunction of monocytes associated with iron overload and age in patients with thalassemia major. Blood 1986; 67:105-9.
4. Tovo PA, Miniero R, Barbera C, Sacchetti L, Saitta M. Serum immunoglobulins in homozygous beta-thalassemia. Acta Haematol 1981; 65:21-5.
5. Costantoulakis M, Trichopoulos D, Augoustaki O, Economidou J. Serum immunoglobulin concentrations before and after splenectomy in patients with homozygous beta-thalassemia. J Clin Pathol 1978; 31:546-50.
6. Corry JM, Marshal WC, Gutthrie LA, Peerless AG, Johnstone RB. Deficient activity of the alternative pathway of complement in beta-thalassemia major. Am J Dis Child 1981; 135: 529-31.
7. Kapadia A, De Sousa M, Markenson AL, Miller DR, Good RA, Gupta S. Lymphoid cell sets and serum immunoglobulins in patients with thalassemia intermedia: relationship to serum iron and splenectomy. Br J Haematol 1980; 45:405-16.
8. Neri A, Brugiatelli M, Iacopino P, Callea V, Ronco F. Natural killer cell activity and T-subpopulations in thalassemia major. Acta Haematol 1984; 71:263-9.
9. Goicoa MA, Estevez ME, Ballart IJ, et al. Impairment of natural killer function in carriers and patients with thalassemia major. Nouv Rev Fr Hematol 1986; 28:75-9.
10. Grady RW, Akbar AN, Giardina PJ, Hilgartner MW, De Sousa M. Disproportionate lymphoid cell subsets in thalassemia major: the relative contributions of transfusion and splenectomy. Br J Haematol 1985; 59:713-24.
11. De Martino M, Rossi ME, Muccioli AT, Vullo C, Vierucci A. Altered T-cell subsets and function in polytransfused beta-thalassemia patients: correlation with sex and age at first transfusion. Vox Sang 1985; 48:296-304.
12. Guglielmo P, Cunsolo F, Lombardo T, et al. T-subset abnormalities in thalassemia intermedia: possible evidence for a thymus functional deficiency. Acta Haematol 1983; 72:361-7.
13. Grady RW. Transfusion related immunologic abnormalities in beta-thalassemia major. Birth Defects 1988; 23:587-93.
14. Speer CP, Gahr M, Schuff-Werner P, Scroter W. Immunologic evaluation of children with homozygous beta-thalassemia treated with desferrioxamine. Acta Haematol 1990; 83:76-81.
15. Uguccioni M, Meliconi R, Nesci S, et al. Elevated interleukin-8 serum concentrations in beta-thalassemia and graft versus host disease. Blood 1993; 81:2252-6.
16. Meliconi R, Uguccioni M, Lalli E, et al. Increased serum concentrations of tumor necrosis factor in beta-thalassaemia: effect of bone marrow transplantation. J Clin Pathol 1992; 45:61-5.
17. Morabito F, Iacopino P, Lentini M, et al. Increased serum levels of soluble interleukin-2 receptors in patients with beta-

thalassemia major. Riv Ital Pediatr 1992;18:272-4.
18. Smith KA. Interleukin-2: inception, impact, and implications. Science 1988; 240:1169-76.
19. Van Snick J. Interleukin 6: an overview. Annu Rev Immunol 1990; 8:253-78.
20. Vilcek J, Lee TH. Tumor necrosis factor: new insights into the molecular mechanisms of its multiple actions. J Biol Chem 1991; 266:7313-6.
21. Kline S. Detection of normal soluble CD4 level in human serum (Abstract). Paper presented at the Fourth Annual Conference on Clinical Immunology and CIS associated Meetings, Arlington, VA, 1989:29.
22. Fujimoto J, Levy S, Levy R. Spontaneous release of the Leu-2 (T8) molecule from human T-cells. J Exp Med 1983; 159: 752-66.
23. Gordon J. The molecules controlling B-lymphocytes. Immunol Today 1987; 8:339-44.
24. Rubin LA, Nelson DL. The soluble interleukin-2 receptor: biology, function and clinical application. Ann Intern Med 1990; 113:619-27.
25. Contu L, Arras M. Molecole di membrana e funzione immunologica. In: Rassu S, ed. Caleidoscopio, Sassari: Medical Systems, 1992, n.73.
26. Janeway CA. The role of CD4 in T-cell activation: accessory molecule or co-receptor? Immunol Today 1989; 10:234-9.
27. Blue ML, Craig KA, Anderson KA, Anderson P, Branton KR Jr, Schlossman SF. Evidence of specific association between class I major histocompatibility antigens and CD8 molecules of human suppressor/cytotoxic cells. Cell 1988; 54:413-21.
28. Veillette A, Brown CE, Coleman FH, et al. The CD4 and CD8 T-cell surface antigens are associated with the internal membrane tyrosine-protein kinase p-56lck. Cell 1988; 55:301-11.
29. Grimfors G, Andersson B, Tullgren O, et al. Increased serum CD8 soluble antigen level is associated with blood lymphocyte abnormalities and other established indicators of a poor prognosis in adult Hodgkin's disease. Br J Haematol 1992; 80:166-71.
30. Pizzolo G, Chilosi M, Semenzato G. The soluble interleukin-2 receptor in haematologic disorders. Br J Haematol 1987; 67:377-80.
31. Scheurich P, Toma B, Ucer U, Pfizenmaier K. Immunoregulatory activity of recombinant tumor necrosis factor (TNF): induction of receptors on human T-cells and TNF mediated enhancement of T-cell responses. J Immunol 1987; 138:1786-92.
32. Cimino G, Amadori S, Cava MC, et al. Serum IL-2, soluble IL-2 receptors and tumor necrosis factor-alpha levels are significantly increased in acute myeloid leukemia. Leukemia 1991; 5:32-5.
33. Kikutani H. Molecular structure of human lymphocyte receptor for immunoglobulin E. Cell 1986; 47:657-65.
34. La Rosa M, Pavone L, Musumeci S, Mollica S, Ronchetti R. Serum IgE in polytransfused thalassemic patients. Vox Sang 1983; 47:384-8.
35. Quintiliani L , Indicone P, Sisti P, et al. Effects of the allogeneic stimulation on B cell function in thalassemic polytransfused patients. Boll Ist Sieroter Milan 1987; 66:316-21.
36. Aglianò AM, Vania A, Gandini O, et al. Post-traanfusion human retrovirus infection in 41 Italian β-thalassemic patients. Haematologica 1992; 77:54-9.
37. Di Marco V, Lo Iacono O, Capra M, et al. α-interferon treatment of chronic hepatitis C in young patients with homozigous β-thalassemia. Haematologica 1992; 77:502-6.