Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 76(1) :99-102, jan./mar. 1981

NOTAS

DYSPROTEINAEMIA IN THE DIFFERENTIAL DIAGNOSIS OF KALA-AZAR

PAMELA LANE MORIEARTY*
RODOLFO DOS SANTOS TEIXEIRA**

Serum protein abnormalities were examined in six kala-azar (KA) patients, six controls with positive immunofluorescence tests with Leishmania donovani *antigens, and six seronegative controls. KA patients were clearly distinguishable from controls by several parameters, including A/G ratio, albumin and globulin levels, IgM and IgG titers, and positive rheumatoid factor (RF) tests. A positive relationship was noted between RF titers and serum levels of IgM. The diagnostic value and possible pathologic significance of serum abnormalities in KA is discussed.*

The differential diagnosis of kala-azar (KA) is frequently difficult, especially in outpatient or field situations. In Brazil, physical signs and symptoms can be similar to those of salmonellosis of protracted course (Teixeira, 1963). In some geographic areas, serological reactions such as indirect immunofluorescence (IFAT) have been found useful (Rezai et al., 1977), but in many areas, serological cross reactions in patients with Chagas' disease or cutaneous leishmaniasis must be considered (Amato Neto, Silva & Camargo, 1977). Another typical feature of KA is a striking dysproteinaemia. The exact etiology of this abnormality is not known, but since it is persistent long after clinical cure (Benallegue et al, 1970; Musumeci, Fischer & Pizzarelli, 1977), it is probably not due to direct stimulation by parasite products, as has been suggested for African trypanosomiasis (Assoku, Tizard & Nielson, 1977). Non-specific elevations of IgG and IgM, with normal levels of IgA, have been reported (Benallegue et al, 1970; Zuckerman, 1975); IgD and IgE levels have not been investigated previously.

To clarify the relation between seropositivity to leishmanial antigens and serum protein abnormalities in the diagnosis of KA, we have studied several parameters in KA patients and in seropositive and seronegative controls.

Sera from six patients with parasitologically confirmed KA and from 12 control subjects of the same age range from the

* Departamento de Imunologia, Instituto Oswaldo Cruz, Caixa Postal 926, 20000 – Rio de Janeiro, RJ, Brasil.

** Departamento de Medicina, Universidade Federal da Bahia, 40000 – Salvador, Bahia, Brasil.

Supported in part by CNPq (Brazil) Grant No. SIP/08-141.

Received for publication on April 11th, 1980.

same endemic area (Jacobina, Bahia, Brazil) were studied. Six control subjects (Control A) had positive (⩾ 1:40) IFAT tests with *L. donovani* antigens, while 6 (Control B) had negative IFAT. Two Control A and 2 Control B subjects also presented either fever, hepatomegaly and/or splenomegaly at the time of examination. None of the controls presented clinical KA during a year's follow-up. For all 18 subjects, serum proteins were analyzed by cellulose acetate electrophoresis; rheumatoid factor (RF) and C-reactive protein (CRP) were tested quantitatively by latex agglutination; antinuclear antibodies (ANA) were tested by indirect immunofluorescence with a human granulocyte substrate; and anti-toxoplasma antibodies were tested by indirect immunofluorescence. In addition, serum levels of IgG, IgA, IgM, IgD, and IgE were quantitated by radial immunodiffusion for KA patients and Control A. Manufacturer's instructions were followed for commercial RF, CRP, toxoplasma, and radial immunodiffusion reagents. All tests were carrried out with non-inactivated serum.

As shown in Table I, though there was some overlap in total serum proteins, KA patients could be clearly distinguished on the basis of A/G rations, albumin and gamma globulin levels. There was no significant difference in levels of alpha 1, alpha 2, or beta globulins. IgG levels were higher in all KA cases, and IgM levels exceeded those of controls in 5/6 cases. No significant differences were apparent in IgA, IgD or IgE levels. ANA tests were uniformly negative. High titers of anti-toxoplasma antibodies (⩾ 1:2,000; > 300 IU/ml) were present in 2 Control A and 2 Control B subjects, but were not associated with serum protein abnormalities which might be confused with those of kala-azar; toxoplasma-antibody positive subjects did not have fever, hepatomegaly or splenomegaly.

CRP was positive in 4 KA patients and in none of the controls, while RF was positive in all KA patients and in no controls. In addition, we found a positive relatioship between RF titers and serum levels of IgM, but not IgG or CRP titers (Table II).

Our findings confirm the value of serum protein analysis in the differential diagnosis of KA, especially in cases in which physical examination and even specific serology may give confusing results. The relation between IgM levels and RF titers is interesting, since the antiglobulin measured by this test is an IgM antibody directed against IgG determinants (Johnson & Faulk, 1976). Though rheumatoid

TABLE I

Range of serum protein values in kala-azar patiente (K A), and in endemic-area controls positive (Control A) and negative (Control B) in the *L. donovani* IFAT

	KA	*Control A*	*Control B*
Total proteins g%	6.45 – 11.30	5.82 – 7.27	6.40 – 7.31
A/G ratio %	0.29 – 0.64*	1.09 – 1.65	1.10 – 1.73
Albumin g%	1.53 – 2.74*	3.50 – 4.28	3.67 – 4.06
γ globulins g%	2.47 – 7.75*	1.00 – 1.87	0.98 – 1.78
IgG IU/ml	283 – 662*	120 – 247	–
IgM IU/ml	241 – 701	102 – 342	–
IgA IU/ml	31 – 158	34 – 158	
IgD IU/ml	0 – 51	0 – 131	–
IgE IU/ml	0 – 5900	0 – 4900	–

*KA values significantly different from controls A and B ($P<0.01$, unpaired ranking test).

TABLE II

Immunoglobulin values, rheumatoid factor (RF), and C-reactive protein (CRP) titers in KA

KA Case	*RF titer*	*IgM IU/ml*	*IgG IU/ml*	*CRP titer*
1	20	241	319	2
2	80	433	543	neg
3	80	494	467	1
4	160	353	283	1
5	160	506	662	neg
6	320	701	423	4

factor-like antiglobulins have long been known to occur in KA, neither the value of this test in field screening, nor the possible pathological significance of the reaction, has been fully explored. Though not pathognomonic for KA, this typically positive test has the attractive features of extreme ease of performance, relatively low cost, and universal availability of reagents. It is also interesting to speculate that antiglobulins might contribute to the persistently raised IgG levels, through a type V or secretory hypersensitivity mechanism (Roitt, 1977), by reacting with lymphocytes or IgG-secreting plasma cells (Gausset et al., 1975). In this context, further information concerning antigenic site specificity of the antiglobulin and the persistence of antiglobulin titers after cure will be relevant.

RESUMO

Foram examinadas alterações de proteínas séricas em seis pacientes com calazar (KA), em seis controles com testes positivos de imunofluorescência tendo como antígeno *Leishmania donovani*, e seis controles seronegativos. Pacientes com KA foram nitidamente distinguíveis dos grupos controles através de vários parâmetros, incluindo a relação A/G, níveis de albumina e globulinas, títulos séricos de IgM e IgG, assim como teste para fator reumatóide (FR) positivo. Uma relação direta foi observada entre os títulos de FR e os níveis séricos de IgM. O valor diagnóstico e o possível significado patológico dessas anormalidades séricas ocorrendo no KA são discutidos.

ACKNOWLEDGEMENTS

We wish to thank Instituto Behring, Hoechst do Brasil, for kindly providing immunodiagnostic kits, and Dr. Rodney Hoff, PAHO/Harvard/Wellcome/UFBa project, Salvador, Brazil, for carrying out IFAT determinations with leishmanial antigens.

REFERENCES

AMATO NETO, V.; SILVA, L.J.D.; CAMARGO, M.E., 1977. Resultados de reações para diagnóstico da doença de Chagas, executadas com soros de pacientes acometidos de leishmaniose visceral. *Rev. Inst. Med. Trop. São Paulo 19* :99-102.

ASSOKU, R.K.G.; TIZARD, I.R.; NIELSON, K.H., 1977. Free fatty acids, complement activation, and polyclonal B-cell stimulation as factors in the immunopathogenesis of African trypanosomiasis. *Lancet ii* :956-959.

BENALLEGUE, A.; IRUNBERRY, J.; GRANGAUD, J.-P.; MAZOUNI, M.; BENABDALLAH, S.; BELAMINE, M.-N., 1970. La dysprotéinémie du kala-azar. *Arch. Françaises Pédiatrie 27* :771.

GAUSSET, P.; DELESPESSE, G.; HUBERT, C.; KENNES, B.; GOVAERTS, A., 1975. In vitro response of subpopulations of human lymphocytes. II. DNA synthesis induced by anti-immunoglobulin antibodies. *J. Immunol. 116* :446-453.

JOHNSON, P.M. & FAULK, W.P., 1976. Rheumatoid factor: Its nature, specificity, and production in rheumatoid arthritis. *Clin. Immunol. Immunopathol. 6* :414-430.

MUSUMECI, S.; FISCHER, A.; PIZZARELLI, G., 1977. Dysproteinaemia in kala-azar. *Trans. Royal. Soc. Trop. Med. Hygiene 71* :176-177.

REZAI, H.R.; BEHFOROUZ, N.; AMIRHAKIMI, G.H.; KOHANTEB, J., 1977. Immunofluorescence and counter immunoelectrophoresis in the diagnosis of kala-azar. *Trans. Royal Soc. Trop. Med. Hygiene 71* :149-151.

TEIXEIRA, R.S., 1963. A febre tifóide de curso prolongado e o calazar (estudo comparativo). *O Hospital (Rio) 63* :1105-1124.

ROITT, I., 1977. *Essential Immunology*, 3rd Ed. Blackwell Scientific Publ., Oxford. p. 183-185.

ZUCKERMAN, A., 1975. Current status of the immunology of blood and tissue protozoa. I. *Leishmania. Exp. Parasitol. 38* :370-400.