TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (1986) 81, 978-980

Autoantibodies in humans with cystic or alveolar echinococcosis

HIROSHI MORI[1], BARBARA WERNLI[1], NIKLAUS WEISS[2] AND RICHARD M. FRANKLIN[1]

[1]*Department of Structure Biology, Biocenter, University of Basel, 4056 Basel, Switzerland;* [2]*Department of Medicine, Swiss Tropical Institute, 4051 Basel, Switzerland*

Abstract

Sera from 16 echinococcosis patients were analyzed with respect to polyclonal B cell activation and autoantibody formation. At least 8 of the sera were from patients who were never in tropical countries and therefore their cases were not complicated by other parasitic diseases. In comparison with a group of 52 healthy controls, these patients had significant levels of antibodies to DNP and haemocyanin, indicators of polyclonal B cell activation. There were also significant differences between control and patient groups with respect to antibodies to dsDNA, histones, actin, vimentin, and desmin. This is the first report of autoantibodies in echinococcosis.

Introduction

Polyclonal B cell activation accompanied by the appearance of autoantibodies is a recognized feature of several parasitic diseases such as visceral leishmaniasis (GALVAO-CASTRO *et al.*, 1984), Chagas disease (SCOTT & SNARY, 1982), and malaria (ADU *et al.*, 1982; MORTAZAVI-MILANI *et al.*, 1984). In Chagas disease both humoral and cellular immunopathological mechanisms have been implicated in the overall pathology of the chronic state (SCOTT & SNARY, 1982). The parasitic factor or factors involved in the complex immunopathologies are now under investigation in several laboratories. We report here yet another parasitic disease, caused by larvae of members of the genus *Echinococcus*, which is accompanied by polyclonal B cell activation and autoantibody formation.

Materials and Methods

Patients and controls

Sera from a total of 16 patients (14 with *E.granulosus*, 2 with *E.multilocularis*) were analyzed. There were 7 male and 9 female patients ranging in age from 12 to 72 years. Of these, 8 had never been to the tropics, 2 were Turkish males working in Switzerland, and one had visited Tibet. Unfortunately no records of any possible visits to tropical countries were available for the remaining 5 patients. Nevertheless, there was no evidence that any patient was suffering from any other parasitic or other infectious disease at the time the serum samples were collected. All of the patients were positive for Epstein-Barr virus (EBV) as determined by viral capsid antigen (VCA) and Epstein-Barr nuclear antigen (EBNA) titres. The control sera were from a group of Caucasian normal controls (CNC) selected from laboratory personnel including 32 males and 20 females ranging in age from 20 to 54 years. Of these, 4 were EBV-negative. All were healthy at the time of bleeding and none had been in tropical countries for at least a year before bleeding.

ELISA procedure

The protocol for ELISA tests was modified from that described by VAINIO *et al.* (1983). Microtest plates (A/S Nunc, Raskilde, Denmark) were coated with appropriate amounts of the purified antigens. Most of the antigens used have been described (VAINIO *et al.*, 1983). Dinitrophenylated-bovine serum albumin (DNP-BSA) was prepared in our own laboratory and its ELISA readings were compared with BSA alone. Haemocyanin was *Limulus polyphemus* haemocyanin (Sigma Chemical Co, St Louis, Missouri 63178, USA). Appropriate dilutions of sera were incubated for 2 hours at 37°, the second antibody was peroxidase-labelled goat anti-human Ig (Nordic Immunological Laboratories, Tillburg, The Netherlands). Absorbances (A_{492}) in the individual wells were read with an automatic photometer (Titertec Multiskan, Eflab OY, Helsinki, Finland) interfaced with an Apple IIc computer. Data files stored on floppy disks were processed and analyzed statistically using our own package of statistical programs.

Results

The raw data presented in Table 1 are the mean values of A_{492} ($\pm$ SD) of the appropriate serum dilutions (always the same dilution for the control and patient groups), rounded off to two significant figures. Background A_{492} has not been subtracted and is also shown in Table 1. The background in the absence of serum always has a considerably lower A_{492} than the serum samples but the absolute value is quite high in the case of keratin. Although there is a clear trend to higher A_{492} average values in the patient group, we cannot make any judgement concerning quantitative differences between control and patient groups without further statistical analyses. Analysis using CNC standard deviation (SD) units (cf. VAINIO *et al.*, 1983 for a similar, but not identical, analysis), however, indicates that the average CNC SD units are all greater than zero for the patient series and the average values for haemocyanin, dsDNA, histones, actin, keratin, laminin, and collagen type I are all greater than 1 (Table 1). The SDs for the CNC SD units do fluctuate very strongly, however. As we are dealing with small samples, we applied the Wilcoxon rank test to detect differences between the two groups. We ranked both A_{492} and CNC SD units from lowest to highest and compared the hypotheses that the two groups (CNC and patients) were the same or different. As a measure of the difference we used Z_0 and present the results in Table 2 along with the corresponding p-values. Differences between the two groups may be seen, with the patient group always having higher values (except for ssDNA, tubulin, laminin, and collagen type I, where no differences were detected). The differences for haemocyanin, dsDNA, histone, actin, and vimentin were significant at the $p<0{\cdot}005$ level; that for keratin at the $p<0{\cdot}01$ level; and those for DNP and desmin at the $p<0{\cdot}05$ level. The same results were obtained ranking A_{492}

Table 1—ELISA tests for polyclonal B cell activation and autoantibodies in controls and echinococcosis patients

Antigen	*CNC(A_{492})*[1]	*Patients(A_{492})*[2]	*Background(A_{492})*[3]	*Patients*[4]
DNP	·26 ± ·04	·29 ± ·54	·15 ± ·16	·77 ± 1·19
Haemocyanin	·90 ± ·25	1·35 ± ·52	·26 ± ·01	1·80 ± 2·09
dsDNA	·99 ± ·28	1·38 ± ·44	·24 ± ·01	1·41 ± 1·60
ssDNA	·75 ± ·19	·88 ± ·52	·24 ± ·03	·60 ± 2·77
Histone	·45 ± ·10	·87 ± ·30	·15 ± ·01	4·98 ± 2·98
Actin	·92 ± ·17	1·38 ± ·59	·19 ± ·01	2·71 ± 3·47
Tubulin	·57 ± ·19	·60 ± ·19	·14 ± ·01	·19 ± 1·03
Desmin	·43 ± ·14	·52 ± ·18	·15 ± ·02	·60 ± 1·28
Vimentin	·43 ± ·16	·56 ± ·24	·17 ± ·01	·85 ± 1·45
Keratin	1·21 ± ·18	1·49 ± ·42	·60 ± ·03	1·51 ± 2·26
Laminin	·38 ± ·18	·55 ± ·52	·15 ± ·01	1·12 ± 3·55
Collagen Type I	·36 ± ·13	·53 ± ·30	·15 ± ·01	1·29 ± 2·31

1) Mean A_{492} ± SD of the group of 52 Caucasian normal controls (CNC). Background values (in the absence of serum) are given in (3) but are not subtracted from (1).
2) Mean A_{492} ± SD of the group of 16 echinococcosis patients. The comment concerning background values given under (1) is applicable here as well.
4) Mean CNC SD units ± SD of the 16 patients; CNC SD units = (mean A_{492} patients - mean A_{492} CNC)/(SD of A_{492} CNC).

Table 2—Quantitation of the Wilcoxon rank test

Antigen	Z_O[(1)]	p[(2)]
DNP	2·1687	<0·05
Haemocyanin	3·4121	<0·005
dsDNA	3·3676	<0·005
ssDNA	·2241	*
Histone	4·4402	<0·005
Actin	3·8169	<0·005
Tubulin	·8458	*
Desmin	2·0530	<0·05
Vimentin	2·8410	<0·005
Keratin	2·7181	<0·01
Laminin	1·1494	*
Collagen type I	1·6988	*

(1) Z_o = U-U_R/σ where U is the rank value of the group of 16 echinococcus patients and U_R is the expected rank value assuming no difference in A_{492} between the CNC and patient groups; σ is the standard deviation as defined by Wilcoxon.
(2) p is the probability value (based on the unit normal distribution) for the difference hypothesis.
* means there is no significant difference between the two groups.

and CNC SD units. Correlation coefficients were then calculated for patient antibodies in those cases where significant differences could be found between the CNC and patient groups (Table 3). High correlations ($p<0·01$ level) were found for anti-DNP and all other antibodies except anti-DNA. Anti-DNA could be correlated only with anti-histone at the $p<0·01$ level and anti-histone only with anti-DNP and anti-DNA. All other antibodies correlated with each other and with anti-DNP and anti-haemocyanin at the $p<0·01$ confidence level.

Discussion

Little is known concerning immune mechanisms in cystic or alveolar echinococcosis and it was quite surprising to find evidence for antibodies to DNP and haemocyanin, indicators of polyclonal B cell activation (GALVAO-CASTRO *et al.*, 1984), in these patients. The polyclonal B cell activation could be correlated with antibodies to cytoskeletal proteins. Antibodies to nuclear antigens (dsDNA and histones) were also found but these were not well correlated with polyclonal B cell activation.

Despite the spatial delimitation of the hyatid cyst, humoral and cellular immune reactions are associated

Table 3—Correlation coefficients for A_{492} in the group of echinococcosis patients

Antigen	*DNP*	*Haemocyanin*	*dsDNA*	*Histone*	*Actin*	*Desmin*	*Vimentin*	*Keratin*
DNP	—	·794**	·608*	·627**	·674**	·806**	·712**	·755**
Haemocyanin	·794**	—	·535*	·378*	·860**	·814**	·818**	·877**
dsDNA	·608*	·535*	—	·715**	·504*	·584*	·602*	·558*
Histone	·627**	·378*	·715**	—	·188	·503*	·357	·297
Actin	·674**	·860**	·504*	·188	—	·805**	·882**	·909**
Desmin	·806**	·814**	·584*	·503**	·805**	—	·872**	·847**
Vimentin	·712**	·818**	·602*	·357**	·882**	·872**	—	·895**
Keratin	·755**	·877**	·558*	·297**	·909**	·847**	·895**	—

** $p<0·01$
* $p<0·05$
No asterisk indicates $p>0·1$ (no correlation)

with such lesions. There is a granulomatous cell proliferation with many lymmphocytes, as well as eosinophils and mast cells, surrounding the parasitic vesicles (WILLIAMS, 1982; VUITTON *et al.*, 1984). At the same time there is a suggestion of severe impairment of the immune system, at least in alveolar echinococcosis. In this disease the percentage and absolute number of B cells and total PBL are lower than in controls. Furthermore, despite a normal percentage of T cells, T cell function is impaired, as measured by the leucocyte migration test (VUITTON *et al.*, 1984). High levels of serum Ig (VUITTON *et al.*, 1984) might be one reflection of the polyclonal B cell activation we report here.

Acknowledgements

Miss U. Pira provided technical assistance and Mrs M.-C. Gauler was our excellent word processor. This work was supported in part by research funds from the City of Basel and by a grant from the Sandoz Stiftung.

References

Adu, G., Gwyn Williams, D., Quakyi, I. A., Voller, A., Anim-Addo, Y., Bruce-Tagoe, A. A., Johnson, G. D. & Holborow, E. J. (1982). Anti-sss DNA and antinuclear antibodies in human malaria. *Clinical and Experimental Immunology*, **49**, 310-316.

Galvao-Castro, B., Sa Ferreira, J. A., Marzochi, K. F., Marzochi, M. C., Coutinho, S. G. & Lambert, P. H. (1984). Polyclonal B cell activation, circulating immune complexes and autoimmunity in human american visceral leishmaniasis. *Clinical and Experimental Immunology*, **56**, 58-66.

Mortazavi-Milani, S. M., Badakere, S. S. & Holborow, E. J. (1984). Antibody to intermediate filaments of the cytoskeleton in the sera of patients with acute malaria. *Clinical and Experimental Immunology*, **55**, 177-182.

Scott, M. T. & Snary, D. (1982). American Trypanosomiasis (Chagas' Disease). In: *Immunology of Parasitic Infections*. (2nd edit.). Cohen, S. & Warren, K. S. (editors). Oxford; Blackwell Scientific Publications, pp. 261-298.

Vainio, E., Lenoir, G. M. & Franklin, R. M. (1983). Autoantibodies in three populations of Burkitt's lymphoma patients. *Clinical and Experimental Immunology*, **54**, 387-396.

Vuitton, D. A., Lasségue, A., Miguet, J. P., Hervé, P., Barak, T., Seillés, E. & Capron, A. (1984). Humoral and cellular immunity in patients with hepatic alveolar echinococcosis. A 2 year follow-up with and without flubendazole treatment. *Parasite Immunology*, **6**, 329-340.

Williams, J. F. (1982). Cestode Infections. In: *Immunology of Parasitic Infections*. (2nd edit.). Cohen, S. & Warren, K. S. (editors). Oxford: Blackwell Scientific Publications, pp. 676-714.

Accepted for publication 27th August, 1985.