Some Immunologic Evaluations of Toxoplasmosis in Iraqi Aborted Females

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Abstract

Forty-eight aborted women (Iraqi Arab Muslims) at the first trimester with a serological evidence of toxoplasmosis were investigated. Two age- and ethnic-matched control groups were included: 40 aborted women due to accidental events (Control I), and 40 unmarried (virgin) women (Control II). The subjects were evaluated for the following parameters: HLA-class I antigens (A, B and Cw), blood groups, total and differential counts of leukocytes, lymphocyte subpopulations (CD3+, CD4+ and CD20+ cells), phagocytosis of heat-killed yeast (phagocytic index and NBT index), and total serum levels of immunoglobulins (IgA, IgG and IgM) and complement components (C3 and C4). The HLA-A2 and -Cw8 antigens were significantly increased in the patients, while A3 antigen was significantly decreased. Blood group phenotypes in patients and controls also showed significant variations. The total and differential counts of leukocytes showed no significant differences between patients and controls, with the exception of lymphocytes, which showed a significant decreased count in the patients compared to control II. However, the lymphocyte subpopulations showed a significant increased percentage in patients. The phagocytic index was approximated in patients and controls, while NBT index was significantly decreased. Total serum level of IgG was significantly increased in the patients, while IgA, IgM, C3 and C4 levels did not maintain such variation.

Introduction

Toxoplasmosis is a parasitic disease that occurs in humans due to the intracellular protozoan Toxoplasma gondii. Accidental ingestion of oocysts shed into the environment by cats, or tissue cysts contained in undercooked meat are of a major concern in the disease occurrence (1). Toxoplasma infection may cause no clear symptoms, or a mild illness is experienced by the patient. This is reasoned by the fact of healthy innate and adaptive immune systems that are able to control the infection, and maintain the parasite at a chronic stage (2). As T. gondii is an opportunistic parasite, a re-emergence of the chronic stage to progress to an acute stage is mostly associated with defects in the elements of the immune system, with special reference to lymphocytes (3, 4). A good example of such generalization is the AIDS patients, in which the toxoplasmosis risks the patients for a serious morbidity (5, 6). Other factors may also be involved in the re-emergence of toxoplasmosis, and immunogenetic predisposition may landmark the subject. In this respect, alleles of the human major histocompatibility complex (HLA system) are important elements, not only in toxoplasmosis, but also in many other viral, bacterial and parasitic infectious diseases (7, 8, 9).

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In pregnant infected women, T. gondii can be transmitted vertically from mother to fetus (congenital toxoplasmosis), a phenomenon that may lead to either congenital abnormalities (i.e. hydrocephaly), or termination of pregnancy, and in both cases, immunological deviations have been suggested (10). The latter outcome (aborted women) was the main focus of the present investigation, with the aim to evaluate the role HLA-class I polymorphism, blood groups and other immunological parameters in the aetiopathogenesis of toxoplasmosis.

Materials and Methods

Forty-eight aborted women (Iraqi Arab Muslims) at the first trimester with a serological evidence of toxoplasmosis were investigated. Two age- and ethnic-matched control groups were included: 40 aborted women due to accidental events (Control I), and 40 unmarried (virgin) women (Control II). The abortion was carried out at the Department of Gynecology of the Al-Yarmouk Teaching Hospital (Baghdad), during the period July 2002-February 2003. The aborted subjects were investigated 48 hours post-surgical operation.

To exclude other factors that may be considered as risk factors for abortion and/or immunological abnormalities, the women were tested for syphilis by V.D.R.L. test (negative), and Rh factor by anti-D antibody (positive). The subjects also had normal ranges of thyroid hormones (TSH, T3 and T4), and no symptoms of hypertension and diabetes mellitus were recorded.

Ten milliliters of venous blood were drawn from each subject. The blood samples were evaluated for the following parameters: HLA-class I antigens (A, B and Cw), blood groups, total and differential counts of leukocytes, lymphocyte subpopulations (CD3+, CD4+ and CD20+ cells), phagocytosis of heat-killed yeast (phagocytic index and NBT index), and total serum levels of immunoglobulins (IgA, IgG and IgM) and complement components (C3 and C4). Details of laboratory methods are presented by references 11, 12, 13 and 14.

The results were statistically analysed by Fisher's exact probability (HLA antigens), Chi-square test (blood groups) and the least significant difference (LSD) between means (other parameters). The HLA polymorphism was further presented in terms of relative risk (RR), etiological fraction (EF) and preventive fraction (PF) (11).

Results

1. HLA-Class I Polymorphism: Comparisons between patients and the two control groups (I and II) revealed deviations of three antigens, which were A2, A3 and Cw8 (Table 1). The antigen A2 was significantly increased in the patients (54.2 vs. 27.5 and 30%, respectively), while A3 showed a significantly decreased frequency (8.3 vs. 40 and 37.5%, respectively). The antigen Cw8 also showed an increased frequency in the patients (33.3 vs. 15 and 12.5%), but a significant difference was reached when the comparison was made with control II.

2. Blood Groups: The distribution of blood group phenotypes in patients and the two controls was significantly different ($X2 = 14.706$, D.F. = 6, $P< 0.05$) (Table 2). Decreased frequency of blood group (O) in control I was the main contributor of significance.

3. Total and Differential Counts of Leukocytes: Both total and differential counts showed no significant differences between patients and the two control groups, although decreased counts were observed in the patients (Table 3). The lymphocyte count was an exception, and the difference reached a significant level ($P<0.01$), when the comparison was made with the subjects of control II (1928 ± 91 vs. 2676 ± 398 cells/cu.mm.blood).

4. Lymphocyte Subpopulations: Percentage means ± standard errors of

CD3+, CD4+ and CD20+ lymphocytes are given in table 3. As shown in the table, a significantly increased frequency of CD3+ and CD4+ cells (75.81 and 59.03%, respectively) was observed in the patients as compared to control I (40.33 and 30.33%, respectively) or control II (36 and 30%, respectively). The CD20+ lymphocytes also showed an increased percentage (19.43 vs. 17.8 and 15.25%, respectively), but a significant level ($P<0.01$) was observed when the comparison was made between patients and control II.

5. Phagocytosis: Comparisons between patients and the two control groups revealed no significant difference in the phagocytic index of heat-killed yeast (46.53 vs. 51.1 and 48.15%, respectively). However, a highly significant ($P<0.001$) reduction of NBT substance (NBT index) was observed in the patient's phagocytes (43.51 vs. 65.14 and 63.22%, respectively) (Table 3).

6. Total Serum levels of Immunoglobulins and Complement: No significant differences were observed in the serum mean levels of immunoglobulins (IgA and IgM) and complement components (C3 and C4) between patients and controls. The IgG was an exception in this regard, which showed a significantly increased mean in patients (15.01±0.49 gm/L) and control I (15.53±0.03 gm/L), as compared to control II (9.9±1.26 gm/L) (Table 3).

Discussion

The forthcoming findings clearly demonstrate that toxoplasmosis was participating in the immunological deviations that were observed in the aborted women. The main target of such deviations was the cellular immune response. Molecules of the major histocompatibility complex (HLA polymorphism) control the elements of such response, especially at the recognition phase (15). Investigating the polymorphism of HLA-class I molecules revealed deviations in the frequency of three antigens (A2, A3 and Cw8). A2 was in favour of a positive association (RR = 2.76-3.12; EF = 0.35-0.37), and Cw8 shared a similar manner (RR = 3.5; EF = 0.24), while A3 manifested a negative association (RR = 0.14-0.15; PF = 0.32-0.34). Such demonstrations may contradict HLA-toxoplasmosis association studies carried out in other world populations, in which, different HLA alleles (B27, Bw62, Cw3 and DQ3) have shown associations with toxoplasmosis (7,9,16). The discrepancy can be explained in terms of racial differences, and accordingly different HLA alleles may be involved in the pathogenesis of toxoplasmosis in different populations. Otherwise, HLA molecules may have some role in the severity of disease, and evidences in mice and human are in favour of such generalization. Congenic mice cloned with HLA-B27 developed a more severe form of toxoplasmosis than wild animals (17). In human, the antigen Bw62 showed an increased frequency in infants with severe ocular toxoplasmosis, while their mothers did not demonstrate such association (9). Moreover, an association between a class II HLA antigen (DQ3) and the development of hydrocephalus in infants with congenital toxoplasmosis has been recently observed (16). Therefore, further studies are required to scan the role of HLA markers in the aetiopathogenesis of toxoplasmosis, especially at the molecular level with some emphasizes on the type of infection (chronic or acute).

Employing the blood group phenotypes carried out a further examination of the genetic predisposition. Although, the distributions of A, B, AB and O blood groups in patients and the two controls were significantly different, their relationship to toxoplasmosis was not clear. However, two unrelated outcomes may be highlighted. The first, reduced frequency of blood group O in the first control sample, which was aborted women and the cause of the abortion was

accidents. So, can we consider blood group O as a risk factor for abortion in pregnant women, further studies are required to clarify the theme. The second outcome is the role of blood group antigens (A and B) in the aetiology of toxoplasmosis, because summing the three phenotypes (A, B and AB) in one group increases the frequency up to 54.2%, while its counterpart in control II is 35%. It is difficult to explain such finding, which can not be ignored. However, some concern may be deduced, if we consider the fact that the world populations are divided into two groups with respect of their secretor status of blood groups. They are either secretor (78%) or non-secretor (22%) (18). If we pay this establishment some consideration, an important question may be raised; are A and B antigens present in secretions protect the mucosal surfaces of the intestine? This establishment is further strengthen, especially if we consider that IgG anti-*Toxoplasma gondii* antibodies and ABO blood groups were significantly distributed in Cuban blood donors (19). Therefore a further examination of this respect is required.

Investigating the leukocytes, which are the main arm of immune response, revealed a normal total cell count, and such observation may help in deciding whether the disease is acute or chronic. Accordingly, the present aborted women may be ranked at the chronic stage of toxoplasmosis, and their health status may confirm this, because the disease was constructed, and did not bring the attention of the patients or their physicians, and the only candidate of suspicion was abortion. In contrast, the lymphocyte count may be disturbed whether the disease is acute or chronic. The present results confirm this, and the lymphocytes showed a significant reduced count in the *Toxoplama*-infected women (27.9% of the control value); an observation that may highlight a phenomenon of immune suppression, which has also been suggested recently (2, 3, 4).

Functionally, the lymphocytes are better defined in the light of their subpopulations (CD3+, CD4+ and CD20+ cells) (20). The CD3+ lymphocytes showed about 50% increase in the patients compared to both controls, and such finding highlight the importance of these cells in maintain the *Toxoplasma* infection at a chronic stage. Such conclusion is supported by evidences in AIDS patients, in whom the toxoplamosis, if present, represents an acute disease due to the known T-cell functional deficiency (especially CD4+ cells) mediated by the causative virus (5, 6). The CD4+ cells in the present patients were significantly increased and such observation confirms that these cells were activated and maintained a state of chronic infection. In agreement with this scope, it has been demonstrated that excreted-secreted antigens of *T.gondii* play an important role in the stimulation of the host immune system in both acute and chronic infections, and $CD4^+$ T cells specific for these antigens may be involved in the maintenance of long term immunity to *T.gondii* in healthy chronically infected individuals (21).

The CD20+ cells are a further subset of lymphocytes that are mainly involved in the production of immunoglobulins. These cells showed a significant increase when the comparison made with healthy females (Control II), and such increase was paralleled by a significant increase in the serum level of IgG, but not IgA or IgM. Again, these findings confirm the chronic state of toxoplasmosis in the present patients, and evidences in laboratory animals and human support such task (22, 23).

The production of IgG enhances the phagocytic activity of neutrophils and monocytes. The phagocytic index of peripheral blood phagocytes shared a similar profile in patients and the two

control samples, and no significant difference was observed, however, the NBT index contradicted the theme, and a significant reduction was observed in the patients. It is difficult to explain such finding, but it has been demonstrated that monocytes obtained from the brain of toxoplasma-infected mice produce less interferon-γ than corresponding cells in normal mice (24). Recently, it has been suggested that CD40-CD154 interaction regulates interferon-γ-independent mechanisms of host protection through induction of macrophage antimicrobial activity and modulation of TNF-α signaling. Interferon-γ enhances the production of nitric oxide in the phagocytes (25), and if this cytokine is decreased, the NBT index may also consequently decrease. However, it is too early for reaching a firm conclusion unless the cytokines profiles, especially those of Th1 and Th2 cells, are determined.

Table 1: HLA-class I antigens showing significant variations between toxoplasmosis patients and control groups.

Comparisons	Antigens	Patients		Controls		P	RR	EF	PF
		No.	%	No.	%				
Patients *vs.* Control I	HLA-A2	26	54.2	11	27.5	0.01	3.12	0.37	-
	HLA-A3	4	8.3	16	40.0	0.001	0.14	-	0.34
Patients *vs.* Control II	HLA-A2	26	54.2	12	30.0	0.002	2.76	0.35	-
	HLA-A3	4	8.3	15	37.5	0.001	0.15	-	0.32
	HLA-Cw8	16	33.3	5	12.5	0.02	3.50	0.24	-

Number of patients = 48; Number of control I = 40; Number of control II = 40.

P: Probability; RR: Relative risk; EF: Etiological fraction; PF: Preventive fraction.

Table 2: Distributions of blood group phenotypes in toxoplasmosis patients and control groups.

Blood Groups	Patients		Control I		Control II	
	No.	%	No.	%	No.	%
A	16	33.3	14	35.0	8	20.0
B	8	16.7	12	30.0	4	10.0
AB	2	4.2	4	10.0	2	5.0
O	22	45.8	10	25.0	26	65.0
Total	48	100.0	40	100.0	40	100.0

$X2 = 14.704$; Degrees of freedom = 6; $P < 0.05$.

Table 3: Some cellular and humoral immunological parameters in toxoplasmosis patients and control groups.

Parameters		Mean ± Standard Error		
		Patients (No.= 48)	Control I (No. = 40)	Control II (No. = 40)
Leukocyte Count (cells/cu.mm. blood)	Total	7763±313 **a**	8314±573 **a**	7800±1245 **a**
	Neutrophils	5339±285 **a**	5514±470 **a**	4695±810 **a**
	Lymphocytes	1928±91 **a**	2194±168 **ab**	2676±398 **b**
	Monocytes	287±26 **a**	293±35 **a**	267±56 **a**
	Esinophils	143±20 **a**	183±26 **a**	173±30 **a**
Lymphocytes (%)	CD3	75.81±1.61**a**	40.33±0.55 **b**	36.00±1.55 **b**
	CD4	59.03±2.42**a**	30.33±0.76 **b**	30.00±2.13 **b**
	CD20	19.43±0.66 **a**	17.80±0.48 **ab**	15.25±0.55 **b**
Phagocytosis (%)	Phagocytic Index	46.35±4.42 **a**	51.10±3.21 **a**	48.15±2.98 **a**
	NBT Index	43.51±3.23 **a**	65.14±2.50 **b**	63.22±1.54 **b**
Total Serum Level (gm/L)	IgA	2.53±0.14 **a**	2.47±0.21 **a**	2.06±0.34 **a**
	IgG	15.01±0.49 **a**	15.53±2.03 **a**	9.90±1.26 **b**
	IgM	1.74±0.21 **a**	1.32±0.07 **a**	1.59±0.29 **a**
	C3	1.67±0.06 **a**	1.96±0.07 **a**	1.63±0.43 **a**
	C4	0.34±0.01 **a**	0.29±0.02 **a**	0.31±0.06 **a**

In the same raw; Different letters: Significant difference between means.
Similar letters:No significant difference between means.

References:

1. Kasper, L. H. (1998) Toxoplasma infection. In: *Harrison's Principles of Internal Medicine*, 4th Ed., Edited by A. S. Fauci, K. J. Lasselbacher and J. D. Wilson. McGraw Hill Publications, New York. pp. 1197-1202.

2. Alexander, J. and Hunter, C. H. (1998) Immunoregulation during toxoplasmosis. *Mol. Immunol.*, **70**: 81-102.

3. Denkers, E. Y. (1999) T lymphocyte-dependent effector mechanisms of immunity to *Toxoplasma gondii*. *Microbes Infect.*, **1**: 699-708.

4. Yap, G. S. and Sher, A. (1999) Cell-mediated immunity to *Toxoplasma gondii*: initiation, regulation and effector function. *Immunobiology*, **201**: 240-247.

5. Garly, M. L., Petersen, E., Pedersen, C., Lundgren, J.D. and Gerstoft, J. (1997) Toxoplasmosis in Danish AIDS patients. *Scand. J. Infect. Dis.*, **29**: 597-600.

6. Ayisi, N. K., Wiredu, E. K., Sata, T., Nyadedzor, C., Tsiagbe, V.K., Newman, M., Cofie, C. N. and Taneguchi, K. (1997) T-lymphocytopaenia, opportunistic infections and pathological findings in Ghanaian AIDS patients and their sexual partners. *East. Afr. Med. J.*, **74**: 784-791.

7. Tiwari, J. L. and Terasaki, P. I. (Editors) (1985) *HLA and Disease Associations*. Springer, New York.

8. McLeod, R., Buschman, E., Arbuckle, L. D. and Skamene, E. (1995) Immunogenetics in the analysis of resistance to intracellular pathogens. *Curr. Opin. Immunol.*, **7**: 539-552.

9. Meenken, C., Rothova, A., de-Waal, L. P., van-der-Horst, A. R., Mesman, B. J. and Kijlstra, A. (1995) HLA typing in congenital toxoplamosis. *Br. J. Opthalmol.*, **79**: 494-497.

10. Prigione, I., Chiesa, S., Taverna, P., Ceccarelli, R., Frulio, R., Morandi, F., Bocca, P., Cesbron-Delauw, M. F. and Pistoia, V. (2005) T cell mediated immune responses to *Toxoplasma gondii* in pregnant women with primary toxoplasmosis. *Microbes Infect.*, **8**: 552-560.

11. Ad'hiah, A. H. (1990) *Immunogenetic Studies in Selected Human Diseases*. Ph.D.

Thesis, University of Newcastle upon Tyne, England.

12. Sood, R. (Editor) (1985) *Haematology for Students and Practitioners*. Jaypee Brothers, India.

13. Myers, R. L. (Editor) (1995) *Immunology, a Laboratory Manual*, 2nd Edition. Wm C. Brown Communications, Inc. U.S.A.

14. Harlow, E. and Lane, D. (1996) *Antibodies, a Laboratory Manual*. Cold Spring Harbor Laboratory, U.S.A.

15. Unanue, E. R. (1995) Antigen processing and presentation. In: *Samter's Immunologic Diseases*, 5th Edition, Edited by M. M. Frank, K. Frank Austen, H. N. Claman and E. R. Unanue. Little, Brown and Company, U.S.A. pp. 87-99.

16. Mack, D. G., Johnson, J. J., Robert, F. and Robert, C. W. (1999) HLA-class II genes modify outcome of *Toxoplasma gondii* infection. *Int. J. Parasitol.*, **5**: 1351-1358.

17. Brown, C. R., David, C. S., Khare, S. J. and McLeod, R. (1994) Effects of human class I transgenes on *Toxoplasma gondii* cyst formation. *J. Immunol.*, **152**: 4537-4541.

18. Nora, J. J. and Fraser, F. C. (Editors) (1989) *Medical Genetics: Principles and Practice.* 3rd Edition, Lea and Febiger, Philadelphia. pp. 399.

19. Lopez, R., Fano, R., Contreras, R. and Font, L. (1993) IgG antibodies against *Toxoplasma gondii* in Cuban blood donors. *Rev. Latinoam. Microbiol.*, **35**:207-210.

20. Lydyard, P. and Grossi, C. (1998) Cells involved in the immune response. In: *Immunology*, 5th edition, Edited by I. Roitt, J. Brostoff and D. Male. Mosby International, U.K., pp. 14-30.

21. Prigione, I., Facchetti, P., Lecordier, L., Deslee, D., Chiesa, S., Cesbron-Delauw, M. F. and Pistoia, V. T. (2000) T Cell clones raised from chronically infected healthy humans by stimulation with *Toxoplasma gondii* excretory-secretory antigens cross-react with live tachyzoites: characterization of the fine antigenic specificity of the clones and implications for vaccine development. *J Immunol.*, **164**:3741-3748.

22. Villavedra, M., Carol, H. and Nieto, A. (1998) Evolution of IgG antibody response against *Toxoplasma gondii* tissue cyst in acute and chronic human infections. *Rev. Inst. Med. Trop. Sao. Paulo.*, **40**: 77-84.

23. Petersen, E., Borobio, M. V., Guy, E., Liesenfeld, O., Meroni, N., Naessens, A. and Spranzi, E. (2005) European multicenter study of the LIAISON automated diagnostic system for determination of *Toxoplasma gondii*-specific immunoglobulin G (IgG) and IgM and the IgG avidity index. *J. Clin. Microbiol.*, **43**: 1570-1574.

24. Reichmann, G., Villegas, E. N., Craig, L., Peach, R. and Hunter, C. A. (1999) The CD28/B7 interaction is not required for resistance to *Toxoplasma gondii* in the brain but contributes to the development of immunopathology. *J. Immunol.*, **163**: 3354-3362.

25. Subauste, C. S. and Wessendarp, M. (2006) CD40 restrains in vivo growth of *Toxoplasma gondii* independently of gamma interferon. *Infect Immun.*, **74**:1573-1579.

بعض التقييمات المناعية لداء المقوسات في عراقيات مجهضات

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الخلاصة:

درست ثمان وأربعون إمرأة مُجهضة (مسلمات عربيات عراقيات) في الثُلث الأول من الحمل مَع وجود دليل مصلي على خمجهن بداء المقوسات. كما شملت الدراسة مجموعتي سيطرة مناظرة بالعمر والعرق: 42 إمرأة مُجهضة بسبب أحداث عرضية (السيطرة الأولى)، و40 أمرأة غير متزوجة (عذراء) (السيطرة الثّانية). قُيّمتْ العينات للعوامل المناعية التالية: مستضدات التطابق النسيجي الصنف الأول (Cw, B, A) ومجاميع الدم والعد الكلي والتفريقي لخلايا الدم البيض والمجاميع الثانوية للخلايا اللمفية (CD20+, CD4+, CD3+) وبلعمة الخميرة المقتولة بالحرارةَ (دليل البلعمة ودليل NBT) والمستوى المصلي الكلي للكلوبيولينات المناعية (IgA و IgG وIgM) ومكوّنات المتمم (C3وC4). أزداد تكرار المُسْتَضّدين Cw8, A2 بشكل معنوي في المرضى، بينما أنخفض معنويا تكرار المُسْتَضد A3 ِ. كما أظهرت مجاميع الدم توزيعات متباينة معنويا في المرضى والسيطرة. لم يظهر العد الكلي والتفريقي لخلايا الدم البيض أي إختلافات معنوية بين المرضى والسيطرة، بإستثناء الخلايا اللمفية، والتي أنخفض عددها معنويا في المرضى مقَارنةَ بالسَيْطَرَة الثّانية. في حين ارتفعت النسبة المئوية للمجاميع الثانوية للخلايا اللمفية في المرضى بصورة معنوية. أما دليل البلعمة فقد تقأرّبت قيمةَ في المرضى والسيطرةِ، بينما أنخفض دليل NBT بشكل ملحوظ. كما أرتفع المستوى المصلي للكلوبيلين المناعي IgG بشكل ملحوظ في المرضى، بينما لم يظهر IgA وIgM وC4 وC3 مثل هذا الإختلافِ.