Blood lymphocytes from ankylosing spondilitis patients fail to induce disease-specific cytotoxic T lymphocytes.

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Blood Lymphocytes from Ankylosing Spondylitis Patients Fail to Induce Disease-Specific Cytotoxic T Lymphocytes

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ABSTRACT *The intriguing observation made by Geczy et al [1] showing the possibility of generating specific ankylosing spondylitis–cytotoxic T lymphocytes by presenting HLA-B27 AS^+ cells as antigen-specific stimulator cells prompted us (by using Geczy's approach) to identify cytotoxic T lymphocytes specific for this apparent $B27^+AS^+$ target structure. Peripheral blood mononuclear cells (PBMC) of 21 healthy $B27^+$ individuals were stimulated in primary and in short-term cultures with PBMC of an HLA identical sibling suffering from definite AS ($n = 12$). In addition PBMC in vitro modified by Geczy bacterial products from two healthy $B27^+$ individuals were used to stimulate $B27^+AS^-$ lymphocytes (either autologous or from a healthy HLA identical sibling). Effector cells raised in primary AS^+ versus AS^- and AS^- versus modified B27 mixed lymphocyte culture combinations showed no proliferative nor cytotoxic activity at all. The scarcely observed cytotoxic reactivity of restimulated mixed lymphocyte culture was not restricted to AS^+ $B27^+$ cells. These results demonstrate that PBMC from ankylosing spondylitis patients fail to induce disease specific cytotoxic T lymphocytes and suggest that an ankylosing spondylitis–related modified B27 structure does not exist at least in the patient material tested.*

ABBREVIATIONS

AS	ankylosing spondylitis	MLC	mixed lymphocyte culture
CML	cell mediated lymphocytotoxicity	MLR	mixed lymphocyte reaction
CTL	cytotoxic T lymphocyte	PBL	peripheral blood lymphocytes
LD	limiting dilution	PBMC	peripheral blood mononuclear cells
minor H	minor histocompatibility		

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Received May 31, 1988; revised December 22, 1988.

0198 8859/89/$3.50

INTRODUCTION

The association of HLA B27 with ankylosing spondylitis (AS) still is one of the best examples of the association between human leukocyte antigens (HLA) and disease Either the B27 gene product itself or a disease susceptibility gene product in tight linkage disequilibrium with B27 must account for the observed association between HLA and disease So far no evidence favoring one of the latter possibilities has been presented

It has been reported that antibacterial sera can distinguish lymphocytes of B27 AS patients from B27 healthy blood donors [2–5] These findings suggested an interaction of enteric organisms (or their products) with B27 Independent laboratories have never succeeded in reproducing these results [6–8] although blind workshops confirmed that cells from AS patients could be distinguished from control cells with the Geczy sera [9–11]

The latter observation led to the hypothesis that there might exist an alien or modified structure on AS B27 cells If so the question could be raised whether allogeneic T cells also recognize a B27 associated structure on lymphocytes of AS patients This possibility was examined by Geczy et al [1] who reported the recognition of an AS associated structure by cytotoxic T lymphocytes (CTL) In two independent laboratories we performed similar experiments with an identical genetic design In our hands no detectable proliferative or CTL responses were induced in mixed lymphocyte cultures (MLC) of cells from healthy individuals stimulated with cells from HLA identical AS patients

MATERIALS AND METHODS

Cells Peripheral blood lymphocytes (PBL) were isolated from defibrinated blood of randomly selected healthy B27 blood donors of definite AS patients (according to the New York criteria) and of healthy B27 siblings who were HLA identical with the AS proband but did not have evidence of sacroiliitis This radiologic sign can be considered as a condition sine qua non for the diagnosis of AS The cells were used fresh or were stored in liquid nitrogen until use Modified B27 AS cells were obtained by incubating cells with the culture filtrate K43 (obtained from A F Geczy) according to the protocol described in Materials and Methods by Geczy et al [1] Control cells were incubated with the culture filtrate F99

MLC The proliferative activity of effector cells was measured in MLC by incubating 5×10 responder cells with 5×10 irradiated stimulator cells in 200 μl of culture medium in microtiter plates After 5 days of incubation H thymidine was added and 24 hr later the incorporation was measured

CTL The generation of AS specific effector cells and the testing for specific cytotoxic activity using the standard cell mediated lymphocytotoxicity (CML) assay have been carried out according to the protocol described in Materials and Methods by Geczy et al [1]

CTL lines In a limiting dilution (LD) culture system CTL lines were generated Graded numbers of responder PBL (10^3–3×10^5) were stimulated with 10^5 irradiated PBL from an HLA identical AS affected sibling in 200 μl of culture medium containing interleukin 2 (IL 2) (40 units/ml) After 6 days of incubation two samples of 50 μl of the cell culture of each well were tested for specific cytotoxicity against stimulator and another AS B27 target cell in the CML

assay The remaining cells in individual wells were restimulated with 10^5 stimulator cells in 100 μl fresh medium Another screening for cytotoxicity was performed at day 12 Those cell cultures that were found to be positive on both target cells were transferred into wells containing 1 ml of culture medium and were restimulated with 10^6 stimulator cells at days 13 and 19 At days 15 22 and 24 1 ml of medium containing IL 2 was added Cell cultures with abundant proliferation were divided The CTL lines were tested for AS specific B27 associated cytotoxicity at day 25 against the stimulator cells and against two additional AS B27 and two AS B27 target cells at an effector to target ratio of 10 1 (the number of target cells was 4×10^3)

RESULTS

The cytotoxic activities of effector cells generated in 21 AS /AS HLA identical sibling responder/stimulator pairs within 10 families and in three AS /AS HLA class I identical (B27 subtype–matched) class II–nonidentical unrelated combinations (see Tables 1 and 2) were tested in the CML assay In one HLA identical sibling pair (both healthy B27 individuals) effector cells were generated using stimulator cells that were modified with either a K13 or F99 (control) culture supernatant Furthermore cells from one randomly selected healthy B27 blood donor were stimulated with autologous cells either K13 or F99 modified Cytotoxicity was measured after 6 days of culturing as well as after restimulation of CTL on day 14 of culture as described in Tables 1 and 2

In combinations of HLA identical siblings (AS AS) the MLC was always found to be negative (proliferation was only tested in the combinations from Amsterdam) In none of the HLA identical AS /AS sibling combinations did the CML assay show specific lysis above that of the medium control of the stimulator or third party AS B27 target cells Cells from the same responders stimulated against HLA class I– and class II–mismatched cells showed good proliferation and effectively lysed (30–60% ^{51}Cr release) stimulator target cells No specific lysis of AS target cells was found in the AS /AS combinations of HLA class I– identical unrelated individuals differing in class II antigens although all had a positive MLC Moreover in none of the CTL raised against stimulator cells modified with bacterial culture filtrates was AS specific cytotoxicity detected Representative results of the experiments are given in Figure 1

Eckolf and Shaw [12] described an LD culture system as a primary in vitro method to generate HLA restricted CTL specific for minor histocompatibility (minor H) antigens between HLA identical siblings The possibility of producing AS specific CTL in such an LD system was examined From four AS /AS sibling

TABLE 1 Cells used in Amsterdam for the induction of AS specific CTL

Combination	Responder cells	Stimulator cells
Siblings		
HLA [illegible] identical	B27 AS	B27 AS
HLA class II identical	(n 15)	(n [illegible])
Unrelated		
HLA class I identical	B27 AS	B27 AS
HLA class II different	(n 3)	(n 5)

[illegible] target cells [illegible] CTL cell lines [illegible] AS AS siblings were tested as described in *Materials and Methods*

TABLE 2 Cells used in Leiden for the induction of AS specific CTL

Combinations	Responder cells (r c)	Stimulator cells (s c)	Target cells
Siblings			
HLA class I– and class II identical	B27 AS (n 6)	B27 AS (n 6)	Autologous (r c r c)
			Specific s c
			Unrelated B27 AS (n – 1)
			Unrelated B27 AS (n ?)
Sibling[b]			
HLA class I– and class II identical	B27 AS (n 1)	B27 AS K43 modified	Autologous (r c r c)
			Specific s c K43 modified
			Specific s c F99 modified
	Same r c	Same s c F99 modified	Unrelated B27 AS (n 1)
			Unrelated B27 AS (n 2)
Unrelated	B27 AS (n – 1)	Autologous (r c r c K43 modified)	Specific s c K43 modified
			Specific s c F99 modified
			Unrelated B27 AS (n 1)
	Same r c	Autologous (r c r c F99 modified)	Unrelated B27 AS (n ?)

All target cells are used without any mitogenic stimulation (r c 6 days PBL identical to the protocol used by Geezy et al [1]) as well as stimulated for 72 hr with phytohemagglutinin

[b] The responder cell population was stimulated with cells from the HLA identical sibling, admixed with either K43 (modifying factor) or F99 (negative control supernatant) both culture filtrates come from one of the cross reactive organisms (see *Materials and Methods* Geezy et al [1])

The responder cell population has been modified with K43 as well as with F99 and used as stimulator/target cells

pairs T cell lines were generated and tested for AS specific cytotoxicity Some CTL lines were cytotoxic for stimulator target cells some third party AS B27 as well as AS B27 target cells Other T cell lines lysed some third party target cells but were not reactive with the specific stimulator cells None of the cell lines were able to distinguish AS from AS target cells

DISCUSSION

Our experiments carried out in a similar way to those of Geezy et al [1] did not produce the same results Due to variations between laboratories it is difficult to ascertain whether the techniques employed were really identical However we are not aware of important technical differences that might account for the observed discrepancies On the other hand the patient (and control) selection could play a role In the work of Geezy the positive results emerged from two HLA identical AS /AS sibling pairs we have examined 21 such combinations (see Tables 1 and 2) without finding a single positive combination Although there may be some differences in the patients examined it is difficult to accept such a critical degree of discrepancy in the clinical diagnosis of the disease In fact the latter explanation for discrepancy can be excluded due to the presence of the serologically detectable Geezy factor on the cells of Dutch AS patients in previous blind workshops [10 11] Moreover in our experiments no CTL activity was measured in the cultures with K43 modified stimulator cells

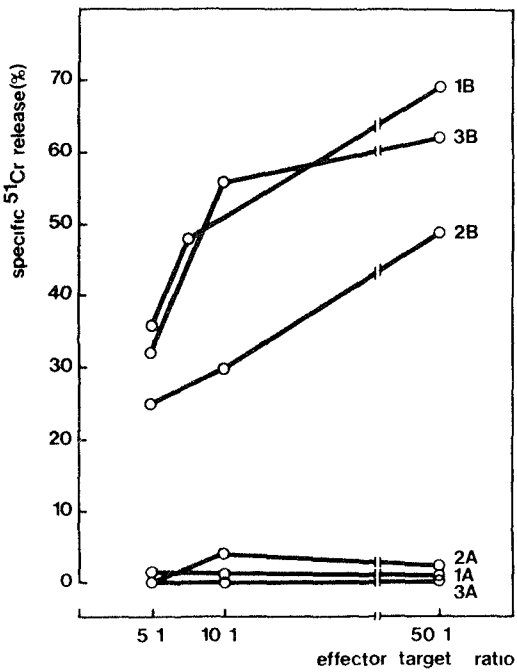

FIGURE 1 Illustrative examples of absence of AS specific cytotoxicity Effector cells were generated in (1) HLA identical AS /AS sibling pairs (2) unrelated HLA class I–identical AS /AS pairs and (3) AS B27 blood donors stimulated with autologous Geezy factor K13 –modified cells AS specific cytotoxic activity was tested on the respective AS stimulator target cells (1A 2A 3A) Allospecific cytotoxic activity of effector cells from the same responders stimulated with allogeneic cells was tested on the respective stimulator target cells (1B 2B 3B)

The framework of the experiments has been extended by efforts to produce uni AS CTL lines in short term cell cultures However no AS specific CTL lines could be detected the cytotoxicity between HLA identical sibling pairs observed in some cases was not restricted to B27 AS target cells and might be due to the recognition of minor H antigens From the results of our control experiments we can conclude that all cells were in good condition Effective CTL could be generated if an inappropriate alloantigen was presented for stimulation All stimulator cells used were able to induce proliferation and could be recognized

by specifically raised effector cells in HLA class I- and class II-mismatched combinations However in our experimental assay the disease and/or B27 associated structure apparently is not antigenic enough for AS specific CTL induction

In conclusion for reasons which are not clear to us we have not been able to induce AS specific CTL by MLC of B27 AS and B27$^+$AS$^+$ cells or by B27 AS cells modified with K43 supernatant culture filtrate The possible modification of MHC antigens on cells with the DR2/DQ1 haplotype of patients with narcolepsy has been studied in a similar genetical set up by Strohmaier et al [13] Narcolepsy is the disease with the strongest HLA association nearly 100% of the patients are DR2/DQ1$^+$ It was reported that no disease related alteration of DR2 and DQ1 molecules on cells of narcolepsy patients could be detected by normal T lymphocytes

Further experiments may elucidate whether disease specific structures do exist on lymphocytes of patients with HLA associated diseases and if so which of the variables are uniquely critical for the activation of disease specific T lymphocytes

ACKNOWLEDGMENT

We thank all patients and their family members who volunteered to participate in this study Professor Dr C P Engelfriet is acknowledged for his critical reading of the manuscript and Professor Dr J J van Rood for helpful discussion We thank Mrs I Gerritsen for secretarial assistance

This study was financially supported by the Dutch Kidney Foundation (grant no C87 693) by the Swiss National Research Fund (grant no 3 912 0 85) and in part by grants from the Dutch Foundation for Medical and Health Research (MEDIGON) and the J A Cohen Institute for Radiopathology and Radiation Protection (IRS)

REFERENCES

1 Geczy AF McGuigan LF Sullivan JS Edmonds JP Cytotoxic T lymphocytes against disease associated determinant(s) in ankylosing spondylitis J Exp Med 164 937 1986

2 Seager K Bashir HV Geczy AF Edmonds J De Vere Tyndall A Evidence for a specific B27 associated cell surface marker on lymphocytes of patients with ankylosing spondylitis Nature 277 68 1979

3 Geczy AF Alexander K Bashir HV Edmonds J A factor in Klebsiella culture filtrates specifically modifies an HLA B27–associated cell surface component Nature 283 782 1980

4 Orban P Sullivan JS Geczy AI Coulits N Bashir HV A factor shed by lymphoblastoid cell lines of HLA B27–positive patients with ankylosing spondylitis specifically modifies the cells of HLA B27–positive normal individuals Clin Exp Immunol 53 10 1983

5 Sullivan JS Geczy AI The modification of HLA B27–positive lymphocytes by the culture filtrates of Klebsiella K43 BTS 1 is a metabolically active process Clin Exp Immunol 62 672 1985

6 Struthers GR Cross reactivity of anti Klebsiella K43 antiserum and lymphocytes from HLA B27–positive patients with ankylosing spondylitis Lancet i 764 1985

7 Georgopoulos K Carson DW Goodacre JA Pain RH A reinvestigation of the cross reactivity between Klebsiella and HLA B27 in the actiology of ankylosing spondylitis Clin Exp Immunol 62 662 1985

8 Singh B Milton JD Woodrow JC Ankylosing spondylitis HLA B27 and Klebsiella A study of lymphocyte reactivity of anti Klebsiella sera Ann Rheum Dis 45 190 1986

9 McGuigan LJ Geezy AI Prendergast JK Edmonds JP Hart HH Bashir HV HLA B27–associated cross reactive marker on the cells of New Zealand patients with ankylosing spondylitis Ann Rheum Dis 45 111 1986

10 Rood JJ van van Leeuwen A Ivanyi P Cats A Breur Vriesendorp BS Dekker Sieys AJ Kuijstra A van Kregten E Blind confirmation of Geezy factor in ankylosing spondylitis Lancet ii 915 1986

11 Geezy AI van Leeuwen A van Rood JJ Ivanyi P Breur BS Cats A Blind confirmation in Leiden of Geezy factor on the cells of Dutch patients with ankylosing spondylitis Hum Immunol 17 239 1986

12 Tekolf WA Shiw S Primary in vitro generation of cytotoxic cells specific for human minor histocompatibility antigens between HLA identical siblings J Immunol 132 1756 1984

13 Strohmaier P Muller Eckhardt G Meier Ewert K Muller Eckhardt C Investigations on the cellular detection of a possible altered self on narcolepsy associated DR2/DQ1 haplotypes Immunobiology 175 261 1987