INFECTION AND IMMUNITY, Sept. 1983, p. 935-941
0019-9567/83/090935-07$02.00/0
Copyright © 1983, American Society for Microbiology
Vol. 41, No. 3

Possible Role of Enteric Organisms in the Pathogenesis of Ankylosing Spondylitis and Other Seronegative Arthropathies

JOHN K. PRENDERGAST,[1]* JOHN S. SULLIVAN,[1] ANDREW GECZY,[1] LINDA I. UPFOLD,[1] JOHN P. EDMONDS,[2] HELEN V. BASHIR,[1] AND ELIZABETH REISS-LEVY[3]

New South Wales Red Cross Blood Transfusion Service,[1] Sydney, New South Wales 2000, and Rheumatology[2] and Microbiology[3] Departments, The St. George Hospital, Kogarah, New South Wales 2217, Australia

Received 24 February 1983/Accepted 10 June 1983

One-hundred eighty-five clinical isolates of *Salmonella* sp., *Shigella* sp., *Escherichia coli*, and *Campylobacter* sp. were tested for their ability to absorb the lymphocytotoxic activity of an antiserum (anti-*Klebsiella* sp. K43) directed against a specific HLA-B27-associated cell surface determinant on the lymphocytes of patients with ankylosing spondylitis (AS). Seven of these isolates (three *Salmonella* sp., two *Shigella* sp., one *E. coli*, and one *Campylobacter* sp.) were found to cross-react with the B27-positive cells of AS patients ($B27^+AS^+$); an *E. coli* organism isolated from the rectal swab of an HLA-B27-negative clinically normal individual also cross-reacted with $B27^+AS^+$ cells. These cross-reactive enteric organisms elaborate a factor (modifying factor) which specifically modifies the B27-positive lymphocytes of normal individuals; this factor is structurally and antigenically related to a functionally similar factor secreted by certain isolates of *Klebsiella* sp. These data suggest that certain enteric organisms share a common determinant which cross-reacts with $B27^+AS^+$ cells. It is suggested that this cross-reactivity is somehow related to an early event in the pathogenesis of AS and possibly of other seronegative arthropathies.

Certain *Klebsiella* sp. antigens cross-react with an HLA-B27-associated cell surface marker on the cells of about 80% of patients with B27-positive ankylosing spondylitis (AS) and 60% of those with Reiter's syndrome or asymmetrical peripheral arthritis (9, 21). These observations imply that certain *Klebsiella* sp., or antigenically related organisms, play an important role in the pathogenesis of these B27-associated seronegative arthropathies (12). However, the finding that HLA-B27-associated reactive arthritis follows infections with enteric organisms such as *Shigella* sp., *Salmonella* sp., and *Yersinia* sp. (1, 10, 19) raises the possibility that a range of enteric bacteria may share determinants with the B27-associated structure on the tissues of patients with AS, reactive arthritis, and other types of B27-positive seronegative arthropathies. Previous surveys of randomly selected cultures of *Klebsiella* sp. indicated that about 8% of *Klebsiella* sp. isolates cross-react with $B27^+AS^+$ lymphocytes (13, 14). We have now broadened this study to include 185 clinical isolates of *Salmonella* sp., *Shigella* sp., *Escherichia coli*, and *Campylobacter* sp. as well as 25 rectal swab isolates of *E. coli* from clinically normal individuals. Cross-reactivity with $B27^+AS^+$ cells was initially assessed by the ability of these organisms to absorb the lymphocytotoxic activity of an anti-*Klebsiella* sp. serum for $B27^+AS^+$ lymphocytes and subsequently confirmed by raising antisera to those isolates which removed the cytotoxic activity of the anti-*Klebsiella* sp. serum. This survey has uncovered three *Salmonella* sp., two *Shigella* sp., one *Campylobacter* sp., and two *E. coli* isolates which were antigenically related to $B27^+AS^+$ lymphocytes. A B27-associated modifying factor (11) elaborated by these organisms shares antigenic determinants with a previously characterized *Klebsiella* sp. isolate (11). The relevance of these findings to the pathogenesis of AS, particularly in the light of recent evidence supporting the specific role of procaryotic genetic material, will also be discussed.

MATERIALS AND METHODS

Patients. HLA-B27-positive and -negative patients were suffering from classical AS as defined by the New York and Rome criteria (4). HLA-B27-positive and -negative healthy controls were staff members or blood donors with no history of joint disease. All patients and controls were tissue typed, using the two-stage National Institutes of Health microlymphocytotoxicity test (18) and sera constantly restandardized by the International Histocompatibility Workshops.

Bacterial cultures. Isolates of *Klebsiella* sp. were obtained as described previously (21, 23). Clinical isolates of *Salmonella* sp., *Shigella* sp., *E. coli*, and *Campylobacter* sp. were supplied by R. Chiew and S. Shankar, Institute of Clinical Pathology and Medical Research, Westmead Centre, Parramatta Hospital, Sydney, Australia. Some shigellae were also received from J. Taplin, Enteric Reference Laboratory, Microbiological Diagnostic Unit, University of Melbourne, Parkville, Victoria, Australia. All cultures were supplied on nutrient agar slopes and routinely subcultured at 2-month intervals. To investigate the possibility that clinically normal individuals harbor *Klebsiella* sp.-cross-reactive organisms, rectal swabs were cultured for *E. coli* on MacConkey agar plates.

Growth and harvesting of bacteria. Before absorption studies, test cultures were grown in nutrient broth (100 ml) for 24 h at 37°C. Cultures were centrifuged at 12,000 × *g* for 30 min. Bacterial pellets were resuspended in 10 ml of nutrient broth and then killed by adding 1 ml of 10% formaldehyde. The Formalin-killed bacteria (10^8 to 10^9/ml) were washed twice with sterile 0.9% NaCl and stored at −30°C.

Preparation of antisera. Rabbits (3 to 5 kg) were immunized with Formalin-killed suspensions of the bacteria as previously described (21). To date, specific cytotoxic antibodies (9) to *Klebsiella* sp. K43 have been raised in 11 different rabbits from our outbred colony. Outbred guinea pigs (400 to 600 g) were also used to raise antisera against some of the Formalin-killed bacterial isolates.

In absorption studies, 0.4 ml of antibacterial serum was added to a pellet of about 5×10^9 Formalin-killed bacteria for 2 h at 4°C. The serum with the bacteria was then centrifuged at 2,000 × *g* for 10 min, the pelleted organisms were discarded, and the serum was absorbed once more with 5×10^9 Formalin-killed organisms for 2 h at the same temperature. After a further centrifugation at 2,000 × *g* for 10 min, the absorbed serum was tested without further dilution. However, it is clear that the serum would have been diluted during the absorption procedure. In a previous study (21) absorbed anti-*Klebsiella* sp., K43 serum, tested at a dilution of 1:8, and undiluted serum gave virtually identical results. The absorption of an antiserum against a "cross-reactive" (i.e., an organism, antiserum to which lyses $B27^+AS^+$ cells) *Klebsiella* sp. isolate (e.g., a particular K43-bearing *K. pneumoniae* isolate) with various bacteria was a preliminary screening procedure designed to identify bacterial isolates which shared antigenic determinants with the cross-reactive *Klebsiella* sp. isolate. For example, a bacterium which could absorb the lymphocytotoxic activity of anti-*Klebsiella* sp. K43 serum for $B27^+AS^+$ cells was considered antigenically related to *Klebsiella* sp. K43.

Lymphocytotoxicity assay. The details of the lymphocytotoxicity assay have been reported (21); however, additional points should be emphasized. Target cell numbers of between 1×10^5 and 8×10^5 per 100 μl of medium have been shown to give reproducible results in the cytotoxicity assay (12, 21; unpublished data). Cytotoxicity values, expressed as percent ^{51}Cr release, are invariably above 50% when target lymphocytes from different AS patients are tested but below 20% (usually <10%) when the cells of clinically normal individuals are studied (9). Furthermore, reproducible (±10%) cytotoxicity values have been obtained when lymphocytes from the same individual are tested on different occasions (9; unpublished data). The influence of different drug treatments on reproducibility of the cytotoxicity assay is not known, but at least six different lymphoblastoid cell lines, derived from $B27^+AS^+$ peripheral blood lymphocytes, invariably gave over 50% ^{51}Cr release, even after 20 generations in culture (2).

Complement, obtained from Pel-Freez Biologicals (Rogers, Ark.), was reconstituted with distilled water shortly before it was added to the incubation mixture of ^{51}Cr-labeled cells and antiserum (21). The complement was used neat, but it was clearly in excess in the reaction since a 1:10 dilution was also effective in mediating lymphocytotoxicity (Table 1). In one series of experiments, 0.5 ml of complement was absorbed with about 5×10^9 Formalin-killed bacteria for 2 h at 4°C. This absorption treatment was repeated, and the twice-absorbed complement was filtered through a 0.2-μm-pore size membrane (Millipore Corp., Bedford, Mass.) and used in the lymphocytotoxicity assay.

The percentage of maximum ^{51}Cr released was calculated as follows: percent ^{51}Cr release = 100 × [(radioactivity released by antiserum − radioactivity released, in absence of antiserum)/(radioactivity released by Nonidet P-40-solubilized cells − radioactivity released in absence of antiserum)].

Assay for modifying factor activity. Peripheral blood lymphocytes or Epstein-Barr virus-transformed lymphoblastoid cell lines from HLA-B27-positive normal individuals (2) were used as targets in the cytotoxicity assay for modification by bacterial culture filtrates (11). Briefly, 5×10^6 to 10×10^6 peripheral blood lymphocytes or lymphoblastoid cell lines were incubated overnight at 37°C in an atmosphere of 5% CO_2 in air in 2 ml of RPMI 1640 (Flow Laboratories, Sydney, N.S.W., Australia) containing 20% human A serum supplemented with penicillin-streptomycin, with or without 100 μl of culture filtrates. The cells were then centrifuged at 800 × *g* for 10 min, resuspended in 1 ml

TABLE 1. Complement, absorbed with cross-reactive and non-cross-reactive isolates, can still mediate specific lymphocytotoxicity for $B27^+AS^+$ lymphoid cells

Antiserum	% ^{51}Cr released from $B27^+AS^+$ lymphocytes with given absorption of complement				
	Nil[a]	*Klebsiella* sp. K43[b]	*S. typhimurium* BTS 69[b]	*Klebsiella* sp. F77[c]	*S. typhimurium* BTS 44[c]
Klebsiella sp. K43	79	80	89	84	96
Salmonella typhimurium BTS 69	85	95	93	88	92
Klebsiella sp. F77	1	1	2	1	2

[a] Similar values (anti-*Klebsiella* sp. K43, 98%; anti-*S. typhimurium* BTS 69, 100%; anti-*Klebsiella* sp. F77, 1%) were obtained with a 1:10 dilution of the complement.

[b] Cross-reactive isolates.

[c] Non-cross-reactive isolates.

of RPMI–10% A serum, and labeled with 50 μCi of ^{51}Cr (0.05 ml; 200 to 500 μCi/μg specific activity; New England Nuclear, Boston, Mass.) for 60 min at 37°C. The washed ^{51}Cr-labeled cells were used in the lymphocytotoxicity assay (11).

Bio-gel P-100 chromatography. Bio-Gel P-100 gel (100 to 200 mesh; Bio-Rad Laboratories, Richmond, Calif.) was equilibrated with phosphate-buffered saline and packed into a glass column (15 by 900 mm). The column was calibrated with the following protein standards (molecular weight in parentheses): bovine serum albumin (67,000), ovalbumin (43,000), pepsin (35,000), trypsinogen (24,000), and lysozyme (14,300). Two-milliliter samples of the culture filtrates were loaded onto the column and run at a flow rate of 3 ml/h at 4°C.) Fractions, 2.5 ml, were collected and assayed for modifying factor activity (23).

Immunoadsorption experiments. Immunoadsorption columns were prepared as previously described (15). In brief, 5 to 10 mg of 40% ammonium sulfate precipitates of serum from rabbits immunized with *Klebsiella* sp. K43 and *Klebsiella* sp. F77 were incorporated into 6 ml of polyacrylamide gel. Two-milliliter samples of concentrated culture filtrate were incubated with 3 ml of each immunoadsorbent for 16 h at 4°C. After the unadsorbed material was washed through the columns with 20 ml of phosphate-buffered saline, the adsorbed material was eluted with 10 ml of 0.1 M glycine-HCl buffer, pH 2.5, containing 0.9% NaCl, and the eluate was neutralized with 1 M Tris solution. The unadsorbed and adsorbed fractions were then concentrated to 2 ml on Amicon CF25 membrane cones and assayed for modifying factor activity.

RESULTS

Clinical isolates of certain enteric organisms remove the cytotoxic activity of an antiserum against a cross-reactive *Klebsiella* sp. isolate. The data presented in Table 2 show that 7 of the 185 clinical isolates tested removed the lymphocytotoxic activity of a rabbit anti-*Klebsiella* sp. K43 serum for B27$^+$AS$^+$ lymphoid cells. These findings suggest that these seven enteric organisms are antigenically related to the cross-reactive *Klebsiella* sp. K43 isolate. To examine the possibility that the removal of cytotoxic antibodies from anti-*Klebsiella* sp. K43 serum by the various clinical isolates was secondary to the "anti-complementary" activity of these isolates, the following experiment was carried out. Complement was absorbed with both cross-reactive (*Klebsiella* sp. K43, *Salmonella typhimurium* BTS 69) and non-cross-reactive (*Klebsiella* sp. F77, *S. typhimurium* BTS 44) isolates. Both absorbed and unabsorbed complement samples were then tested for their ability to mediate the lymphocytotoxic activity of cross-reactive antibacterial sera for B27$^+$AS$^+$ cells. The data (Table 1) show that both absorbed and unabsorbed complement can mediate specific lymphocytotoxicity. It is therefore unlikely that the cross-reactive strains are anti-complementary.

Antisera to cross-reactive enteric organisms lyse B27$^+$AS$^+$ cells. To confirm the observation (Table 2) that the clinical isolates were antigenically related to *Klebsiella* sp. K43, antisera raised in rabbits and in guinea pigs to four of the seven isolates were tested on the lymphocytes of B27-positive and -negative patients and of normal controls. The results (Table 3) indicate that antisera to the four isolates (*Salmonella newport*

TABLE 2. Clinical isolates of *Salmonella* sp. (three), *Shigella* sp. (two), *E. coli* (one), and *Campylobacter* sp. (one) remove the cytotoxic activity of an antiserum against a cross-reactive *Klebsiella* sp. isolate, K43[a]

Absorption	% maximum ^{51}Cr released by target lymphocytes[b]		
	B27$^+$AS$^+$	B27$^+$AS$^-$	B27$^-$AS$^-$
None	85	1	5
Klebsiella sp. K43	0	1	0
Klebsiella sp. F77[c]	74	4	10
Salmonella typhimurium BTS 69	3	3	1
S. typhimurium BTS 111	1	2	3
S. newport BTS 52	0	1	3
Shigella boydii BTS 20	0	1	2
S. flexneri BTS 29	0	NT[d]	0
E. coli BTS 11	0	NT	0
Campylobacter jejuni BTS 13	0	NT	NT
178 other enteric organisms	60–100	0	2

[a] *Klebsiella* sp. K43 is an isolate which cross-reacts with B27$^+$AS$^+$ cells (11). As not all K43-bearing isolates cross-react (12) and to avoid further confusion, this *Klebsiella* sp. isolate will be designated *Klebsiella* sp. K43 BTS 1.

[b] Results are of a representative experiment on the cells of single individuals for each category. All sera were tested undiluted.

[c] *Klebsiella* sp. F77 is a non-cross-reactive isolate which does not cross-react with B27$^+$AS$^+$ cells (23).

[d] NT, Not tested.

TABLE 3. Antisera to four isolates which cross-react with *Klebsiella* sp. K43 also specifically lyse $B27^+AS^+$ lymphocytes

Antiserum	% maximum ^{51}Cr released by target lymphocytes[a]			
	$B27^+AS^+$	$B27^+AS^-$	$B27^-AS^+$	$B27^-AS^-$
Klebsiella sp. K43	92	4	0	0
Klebsiella sp. F77	9	0	0	10
Salmonella newport BTS 52[b]	87	3	10	5
S. typhimurium BTS 69[b]	87	8	8	6
Shigella boydii BTS 20[b]	100	3	13	4
E. coli BTS 11[c]	81	NT[d]	13	NT

[a] Results are of a representative experiment on the cells of single individuals for each category. All sera were tested undiluted.
[b] Antisera to these isolates were raised in rabbits.
[c] Antiserum to this isolate was raised in guinea pigs.
[d] NT, Not tested.

BTS 52, *S. typhimurium* BTS 69, *Shigella boydii* BTS 20, and *E. coli* BTS 11) lysed the lymphocytes of B27-positive but not of B27-negative patients with AS; the cells of B27-positive and -negative clinically normal individuals were also not lysed by any of the antisera. It appears that antisera to these enteric organisms recognize a common antigenic determinant which cross-reacts with $B27^+AS^+$cells. By contrast, antisera raised to non-cross-reactive bacteria such as *Klebsiella* sp. F77 (Table 3) and to some isolates of *E. coli* fail to lyse $B27^+AS^+$ lymphoid cells (J. K. Prendergast, J. S. Sullivan, A. F. Geczy, L. I. Upfold, J. P. Edmonds, and H. V. Bashir, submitted for publication).

Immunoadsorption studies with culture filtrates of cross-reactive enteric organisms. Immunoadsorption columns bearing antisera to *Klebsiella* sp. K43 (cross-reactive isolate) or to *Klebsiella* sp. F77 (non-cross-reactive isolate) were tested for their ability to retain modifying factor activity from culture filtrates of *Klebsiella* sp. K43, *S. newport* BTS 52, *S. typhimurium* BTS 69, *S. typhimurium* BTS 111, and *Shigella boydii* BTS 20. The modifying factor activity of all of the culture filtrates was completely retained by the K43-bearing but not by the F77-bearing immunoadsorbent column (Table 4). Most of the adsorbed modifying factor (from the K43 column) was recovered by elution with 0.1 M glycine-HCl, pH 2.5 (Table 4). The results suggest that the modifying factor elaborated by the four culture filtrates is antigenically related to *Klebsiella* sp. K43.

Bio-Gel P-100 chromatography of concentrated culture filtrates. HLA-B27-related modifying factor was fractionated on a Bio-Gel P-100 column. Samples (2 ml) of 80-fold-concentrated culture filtrates of *S. newport* BTS 52 and *Shigella boydii* BTS 20 were chromatographed on the Bio-Gel column and eluted with phosphate-buffered saline, and the fractions were tested for

TABLE 4. Immunoadsorption of HLA-B27-modifying activity from culture filtrates of cross-reactive enteric organisms

Source of culture filtrate	Serum bound to immunoadsorbent column	% of maximum ^{51}Cr released from $B27^+AS^-$ lymphocytes[a]	
		Unbound material	Bound material
Klebsiella sp. K43	*Klebsiella* sp. K43	3	86
	Klebsiella sp. F77	87	16
Salmonella newport BTS 52	*Klebsiella* sp. K43	7	71
	Klebsiella sp. F77	66	14
S. typhimurium BTS 69	*Klebsiella* sp. K43	8	81
	Klebsiella sp. F77	63	10
S. typhimurium BTS 111	*Klebsiella* sp. K43	9	67
	Klebsiella sp. F77	70	12
Shigella boydii BTS 20	*Klebsiella* sp. K43	10	80
	Klebsiella sp. F77	68	17

[a] Bound (eluate) and unbound (effluent) fractions were tested for modifying activity with anti-*Klebsiella* sp. K43 sera and $B27^+AS^-$ target cells as described in the text.

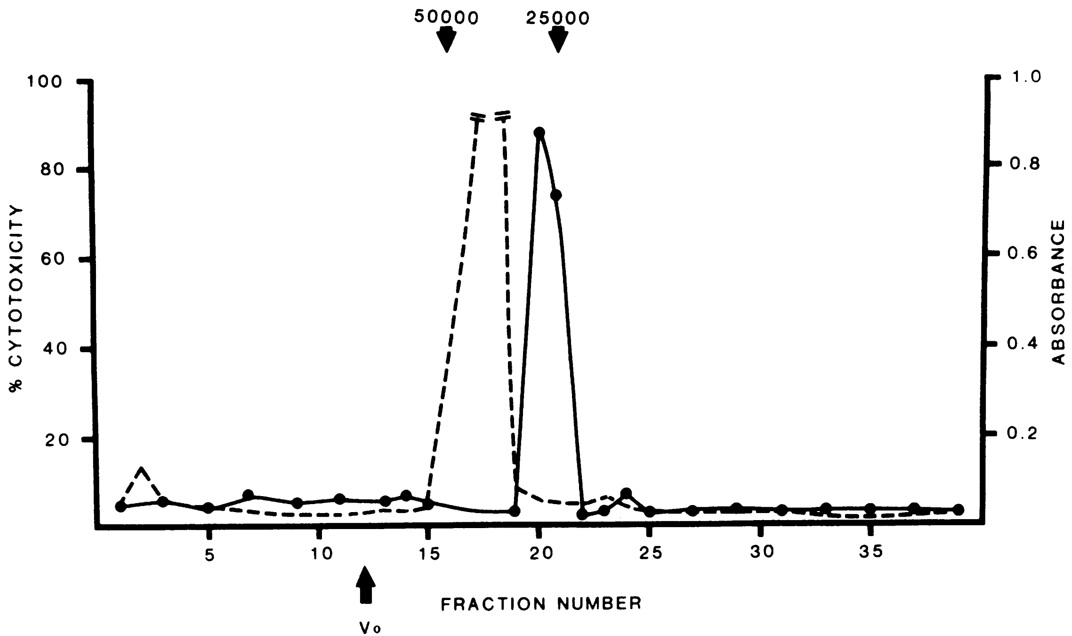

FIG. 1. Bio-Gel P-100 chromatography of concentrated supernatant from *S. newport* BTS 52. Fractions, 2.5 ml, were collected and assayed for protein content (absorption at 280 nm) and for modifying factor activity as described in the text. The elution and activity profiles for *Shigella boydii* BTS 20 were virtually identical to those of *S. newport* BTS 52 (data not shown). Symbols: (- - - -) absorption at 280 nm; (●) modifying factor activity.

their ability to modify $B27^+AS^-$ lymphocytes. The modifying activity of both organisms eluted as sharp peaks with identical elution profiles (Fig. 1). The peaks corresponded to a molecular weight of 26,000 to 30,000 as previously reported by Sullivan et al. (23). It is notable that the modifying activity peaks are well separated from the bulk of "contaminating" protein material.

E. coli **isolated from the bowel flora of a clinically normal individual also cross-reacts with *Klebsiella* sp. K43.** In view of the presence in clinical isolates of cross-reactive enteric organisms, it was reasonable to wonder whether the bowel flora of clinically normal individuals would also include such an organism. *E. coli* was selected as a representative of normal bowel flora. Of 25 rectal swabs, only 1 specimen possessed a cross-reactive isolate of *E. coli* (Table 5). The individual ($B27^-AS^-$) harboring the cross-reactive *E. coli* isolate was further examined for carriage of this organism. Rectal swabs, obtained 2 weeks and then 8 weeks after initial isolation, contained *E. coli* which removed the lymphocytotoxic activity of anti-*Klebsiella* sp. K43 serum.

DISCUSSION

The results of this study have shown that several clinical isolates of pathogenic enteric organisms, in addition to the previously characterized klebsiellae, cross-react with a specific HLA-B27-associated cell surface structure on the cells of patients with AS. These findings imply that a number of serologically unrelated enteric bacteria, including an isolate from a clinically normal HLA-B27-negative individual, share a common determinant or a "public specificity" which is antigenically related to a "modified" HLA-B27 on the cells of AS patients. Furthermore, immunoadsorption experiments as well as Bio-Gel P-100 chromatography indicate that the cross-reactive *Salmonella* sp. (BTS 52) and *Shigella* sp. (BTS 20) isolates both elaborate a B27-associated modifying factor which is identical to or cross-reactive with a

TABLE 5. *E. coli* isolated from a clinically normal individual removes the cytotoxic activity of an antiserum against a cross-reactive *Klebsiella* sp. isolate, K43

Absorption	% maximum ^{51}Cr released by target lymphocytes ($B27^+AS^+$)
None	88
Klebsiella sp. K43	0
Klebsiella sp. F77	80
E. coli isolate[a]	0
24 other *E. coli* isolates	76–98

[a] An *E. coli* isolate recovered 2 weeks and then 8 weeks after initial isolation also removed also removed the cytotoxic activity of *Klebsiella* sp. K43.

functionally similar factor secreted by certain cross-reactive *Klebsiella* sp. organisms.

These findings raise two immediate questions. First, by what mechanism would a minority of serologically unrelated enteric bacteria express on their surface and secrete into their culture medium a common factor capable of modifying a B27-associated cell-surface component? And second, what role might such organisms play in the pathogenesis of AS and related seronegative arthropathies?

The first question may be answered by the preliminary results (F. H. Cameron, P. J. Russell, J. Sullivan, and A. F. Geczy, Mol. Immunol., in press) of a recent study which suggest that an extrachromosomal element, possibly a plasmid, codes for the modifying factor produced by certain cross-reactive *Klebsiella* sp. isolates. It was shown that modifying factor production by a particular cross-reactive *Klebsiella* sp. isolate could be eliminated by "curing" the bacteria of plasmids with acridine orange. In a transconjugation experiment, an *E. coli* isolate, with no detectable plasmids, acquired the ability to produce modifying factor after mating with *Klebsiella*. sp. Although these early results provide only circumstantial evidence, they justify the hypothesis that modifying factor is produced by extrachromosomal elements such as plasmids. Such a mechanism could explain the production of modifying factor by bacteria across a broad spectrum of serologically unrelated organisms.

The role of infectious agents in the pathogenesis of AS is uncertain. Two suggestive findings bear further examination. First, it is well established that AS is related clinically and genetically to other seronegative inflammatory arthropathies such as Reiter's syndrome (25). The latter is a form of reactive arthritis, strongly associated with the presence of HLA-B27 and apparently triggered by any one of three precipitating events: nonspecific urethritis, dysentery caused by certain shigellae or salmonellae, or unidentified factors (5). Although 10 to 15% of patients with Reiter's syndrome ultimately develop AS, it is not known whether this is consequent upon the Reiter's syndrome or a second separate pathological process in an individual with a predisposition to both diseases (16). Second, there are several reports of patients with AS and acute anterior uveitis having a higher incidence of fecal carriage of *Klebsiella* sp. organisms than clinically normal individuals (6–8). Although one group has disputed these findings (24), they shed little light on the mechanism of disease triggering, for it is not clear whether any of these organisms is cross-reactive with the HLA-B27-positive cells from patients with AS or whether any of them generates B27-associated modifying factor.

The possible involvement of microbial organisms or their products in AS may be less conspicuous; that is, the environmental agent may simply act as a focus or a source of genetic material, such as plasmids, which may then interact with HLA-B27-positive cells, as we suggested previously (22). In AS an HLA-associated structure, such as the antigenic determinant shared by several enteric bacteria, may mediate or facilitate the attachment to mucosal surfaces of certain enteric bacteria. This attachment, which is mediated by species-specific microbial adhesins to complementary receptors on the host tissue, is probably essential to the infectious process (3, 17, 20).

These examples aside, there is no clinical evidence to implicate enteric or other infection in the etiology of AS. However, infections cannot be exonerated: the individual's exposure may precede the onset of rheumatic symptoms by many years and, more importantly, the infectious agent may be otherwise nonpathogenic. If the B27-associated cell-surface modification is important in the pathogenesis of AS, the results of the present study provide an important extension to the concept of a role for bacteria. This study demonstrates that a range of bacteria, including an *E. coli* isolate from normal bowel flora, can elaborate a modifying factor identical to that produced by the *Klebsiella* sp. organisms previously studied: this *E. coli* isolate has been shown to persist for a period of at least 2 months. In the light of this observation, it would be important to study the persistence of cross-reactive enteric organisms in the bowel flora of genetically susceptible individuals.

A conclusive statement on the presence, in clinical isolates of enteric organisms, of genes coding for the B27-associated antigenic complex can only be made if hybridization studies can detect such genes. Furthermore, DNA hybridization studies may also enable us to investigate the possibility that plasmid DNA is present in the genome of the cells of patients with AS; these studies are currently in progress.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Kroc Foundation, Santa Ynez, Calif., and by a Philip J. Benjamin Memorial Research Grant, Sydney, N.S.W., Australia.

We thank R. Chiew and S. Shankar for assistance with some of the bacteriological aspects of this work and T. Sonnex for excellent secretarial assistance.

LITERATURE CITED

1. **Aho, K., P. Ahvonen, A. Lassus, K. Sievers, and A. Tiilikainen.** 1974. HL-A27 in reactive arthritis. A study of Yersinia arthritis and Reiter's disease. Arthritis Rheum. 17:521–526.
2. **Alexander, K., C. Edwards, I. S. Misko, A. F. Geczy, H. V. Bashir, and J. P. Edmonds.** 1981. The distribution of a specific HLA-B27-associated cell surface component on the tissues of patients with ankylosing spondylitis. Clin.

Exp. Immunol. **45**:158–164.

3. **Beachey, E. H.** 1981. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Infect. Dis. **143**:325–345.
4. **Bennett, P., and P. Wood (eds.).** 1966. Population studies of the rheumatic diseases, p. 456–457, 478–479. *In* Proceedings of the 3rd International Symposium. Excerpta Medica Foundation, New York.
5. **Calin, A.** 1981. Reiter's syndrome, p. 1033–1046. *In* W. N. Kelley, E. D. Harris, S. Ruddy, and C. B. Sledge (ed.), Textbook of rheumatology, vol. 2. W.B. Saunders Co., Philadelphia.
6. **Eastmond, C. J., H. E. Willshaw, S. E. P. Burgess, R. Shinebaum, E. M. Cooke, and V. Wright.** 1980. Frequency of faecal *Klebsiella aerogenes* in patients with ankylosing spondylitis and controls with respect to individual features of the disease. Ann. Rheum. Dis. **39**:118–123.
7. **Ebringer, R. W., D. R. Cawdell, P. Cowling, and A. Ebringer.** 1978. Sequential studies in ankylosing spondylitis: association of *Klebsiella pneumoniae* with active disease. Ann. Rheum. Dis. **37**:146–151.
8. **Ebringer, R., D. Cawdell, and A. Ebringer.** 1979. *Klebsiella pneumoniae* and acute anterior uveitis in ankylosing spondylitis. Br. Med. J. **i**:383.
9. **Edmonds, J., D. Macauley, A. Tyndall, M. Liew, K. Alexander, A. Geczy, and H. Bashir.** 1981. Lymphocytotoxicity of anti-Klebsiella antisera in ankylosing spondylitis and related arthropathies. Arthritis Rheum. **24**:1–7.
10. **Friis, J., and A. Svejgaard.** 1974. Salmonella arthritis and HL-A27. Lancet **i**:1350.
11. **Geczy, A. F., K. Alexander, H. V. Bashir, and J. Edmonds.** 1980. A factor(s) in *Klebsiella* culture filtrates specifically modifies an HLA-B27-associated cell-surface component. Nature (London) **283**:782–784.
12. **Geczy, A. F., K. Alexander, H. V. Bashir, J. P. Edmonds, L. Upfold, and J. Sullivan.** 1983. HLA-B27, *Klebsiella* and ankylosing spondylitis: biological and chemical studies. Immunol. Rev. **70**:23–50.
13. **Geczy, A. F., and J. Yap.** 1979. HLA-B27, *Klebsiella* and ankylosing spondylitis. Lancet **i**:719–720.
14. **Geczy, A. F., and J. Yap.** 1982. A survey of isolates of *Klebsiella pneumoniae* which cross-react with HLA-B27-associated cell-surface structure on the lymphocytes of patients with ankylosing spondylitis. J. Rheumatol. **9**:97–100.
15. **Geczy, C. L., W. Friedrich, and A. L. deWeck.** 1975. Production and *in vivo* effect of antibodies against guinea pig lymphokines. Cell. Immunol. **19**:65–77.
16. **Good, A. E.** 1979. Reiter's syndrome: long-term follow-up in relation to development of ankylosing spondylitis. Ann. Rheum. Dis. **38**:39–45.
17. **Mackowiak, P. A.** 1982. The normal microbiol flora. N. Engl. J. Med. **307**:83–93.
18. **Mittal, K. K., M. R. Mickey, D. P. Singal, and P. I. Terasaki.** 1968. Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. Transplantation **6**:913–927.
19. **Noer, H.** 1966. An "experimental" epidemic of Reiter's syndrome. J. Am. Med. Assoc. **198**:693–698.
20. **Ofek, I., D. Mirelman, and N. Sharon.** 1977. Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. Nature (London) **265**:623–625.
21. **Seager, K., H. V. Bashir, A. F. Geczy, J. Edmonds, and A. de Vere-Tyndall.** 1979. Evidence for a specific B27-associated marker on the lymphocytes of patients with ankylosing spondylitis. Nature (London) **277**:68–70.
22. **Sullivan, J. S., J. K. Prendergast, and A. F. Geczy.** 1983. The etiology of ankylosing spondylitis: does a plasmid trigger the disease in genetically susceptible individuals? Hum. Immunol. **6**:185–187.
23. **Sullivan, J., L. Upfold, A. F. Geczy, H. V. Bashir, and J. P. Edmonds.** 1982. Immunochemical characterization of *Klebsiella* antigens which specifically modify an HLA-B27-associated cell-surface component. Hum. Immunol. **5**:295–307.
24. **Warren, R. E., and D. A. Brewerton.** 1980. Faecal carriage of *Klebsiella* in patients with ankylosing spondylitis and rheumatoid arthritis. Ann. Rheum. Dis. **39**:37–44.
25. **Wright, V.** 1980. Relationships between ankylosing spondylitis and other spondarthritides, p. 42–51. *In* J. M. H. Moll (ed.), Ankylosing spondylitis. Churchill Livingstone, Edinburgh.