Histocompatibility Antigens and Spondylitis* **

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Dr. Irby: For some time the influence of heredity in ankylosing spondylitis has been fairly well established, but the exact mechanism has not been understood. Although there has been some evidence of familial clustering in peripheral rheumatoid arthritis, studies by Jacox and others in Rochester, New York have indicated 25 of 28 siblings of monozygotic twins to be discordant for rheumatoid arthritis.

The subject of this discussion is HL-A antigens and spondylitis. Since many people do not understand the language of the geneticists, Dr. James Pierce will tell us what we need to know about HL-A antigens. Questions that often arise include: 1) What are HL-A antigens? 2) What is meant by HL-A 27? 3) How does one go about identifying antigenic determinants in tissue typing?

Later we will try to document how some of this material applies to many of the rheumatic diseases in which spondylitis is often a feature. In conclusion, Dr. Macdonald of the Ophthalmology Department will comment on various aspects of anterior and posterior uveitis, how the diagnosis is made, and what the treatment is of each. Uveitis is often a complication of ankylosing spondylitis and

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Reiter's disease. A representative case involving uveitis, arthritis, and the presence of HL-A 27 will be reviewed.

Dr. Edwards: J. C. is a 30-year-old black male who was first seen in the Emergency Room of the Medical College of Virginia Hospitals on September 3, 1973. At that time, he complained of pain in his back and left wrist. He stated that approximately six days earlier, while doing manual labor, he had experienced pain and swelling in his neck. The pain migrated to his left hip, then between the scapulae, and finally into the left wrist. Two weeks prior to these complaints, he described a two day illness characterized by diarrhea from which he recovered with no therapy. Past medical history was significant in that he had been treated five years earlier by his local physician for a urethral discharge. There is no family history of arthritis.

Physical examination at the onset of his illness revealed a T 100.4°F, P 104 per minute, and BP 120/74. There was pain in the area of the cervical spine when his head was deviated to the right or left. In addition there was tenderness to percussion over the midthoracic vertebrae, and a painful, warm, swollen, erythematous left wrist. Also noted was a small papular lesion on his left heel. An arthrocentesis of the wrist was unsuccessful. Laboratory studies included a hemoglobin 16 gm%, WBC's 12,600 (78% polys, 13% lymphocytes, 9% monocytes), erythrocyte sedimentation rate (ESR) 15 mm per hour (Wintrobe), and a urinalysis with a few WBC's and RBC's but no protein, sugar, or acetone. Repeat ESR's have been 30–40 mm per hour. The following tests were either negative or normal: rheumatoid pattern, antinuclear antibody, lupus erythematosus cell preparations, antistreptolysin-O titer, and urine and blood cultures. An intermediate strength PPD was negative at 72 hours. Roentgenographic studies of his chest, cervical spine, thoracic spine, and pelvis were normal. X-rays of the left wrist showed soft tissue swelling only. Scrapings for the papular lesion on his foot grew *Trichophyton mentagrophytes*. HL-A 27 was found to be present using the lymphocyte microcytotoxicity test.

Because of the concern that the patient was suffering from an infectious arthritis, he was treated with antibiotics. There was no response to this therapy and by mid-September he had, in addition to the left wrist, developed painful swelling of his right ankle and pain to palpation in his right wrist. Anti-inflammatory drugs, including salicylates, Butazolidin®, and gold, did not help. Colchicine was likewise ineffective.

On October 8 he presented with an inflamed and swollen right eye. Photophobia was present. An ophthalmological consult found a visual acuity of 20/100 with a raised intraocular pressure in the right eye. The left eye was normal. The etiology for this nongranulomatous anterior uveitis was unknown but felt to be related to the arthritis. Later a posterior uveitis was also noted. Complete resolution of the uveitis occurred over the ensuing six weeks, while the patient was treated with alternate day corticosteroids in a progressively decreasing dose. The arthritis also improved.

Subsequently the patient has complained of left heel pain, yet no objective signs to document the development of bony spurs have occurred. There is no evidence of active synovitis although he does have some limitation of motion of his right ankle and left wrist. He denies any further back pain and is no longer on any medications.

Dr. Irby: We are not at all sure of the exact nature of this gentleman's illness. When the patient was first seen on arthritis rounds, the lesions of his feet were suggestive of keratoderma blennorrhagica. It was felt that we were dealing with a case of ankylosing spondylitis, possibly Reiter's spondylitis, or seronegative rheumatoid arthritis. The latter diagnosis was entertained because of the lack of characteristic x-ray findings in the sacroiliac joints or syndesmophytes on the margins of the lumbar spines. After *Trichophyton mentagrophytes* were cultured from the patient's foot, the only things we had to go on were the clinical picture of arthritis of an asymmetrical nature, past history of urethritis, and uveitis. Dr. Pierce's laboratory determined HL-A 27 to be present, so this gives us an opportunity to present recent data on the new trend in "spondylitisology," that of antigenic determinants by tissue typing for the histocompatibility antigens.

Dr. Pierce: In my discussion of histocompatible antigens, I want to review several things, such as the rapidly changing development of this field, the lymphocytotoxicity test—which provides a basis for detecting these antigens, some comments on the genetics of inheritance of this system of antigens, the nomenclature, a source of constant confusion, and finally, the association of disease states with HL-A antigens.

The field of histocompatibility typing began in 1954, when Dausset, in Paris, discovered that anti-

bodies were present in the sera of patients who had received multiple transfusions, which were directed against leukocytes but not erythrocytes. In 1958 Payne and Rolfe at Stanford University found that women who had had multiple pregnancies had similar antileukocyte antibodies. It was in this same year that the first leukocyte antigen was discovered using antisera, prepared from these patients, which had reacted in common with leukocytes of certain other patients. This particular antigen was HL-A 2 which is the most frequent HL-A antigen found in Caucasians, being present in over 50% of a random population. A significant advance was made in 1964, when Terasaki devised a microtest for detecting these antigens. The advantage of the microtest over the macrotest is that one thousand rather than ten tests can be performed on one milliliter of serum. A series of international workshops were held in the late 1960's. In Torino in 1967, it was first reported that the HL-A antigens were inherited in a Mendelian dominant fashion from parent to child. The discovery that there are two HL-A loci relatively close together on an autosomal chromosome was made in 1970 at the Los Angeles workshop.

The lymphocyte microcytotoxicity test detects surface antigens of lymphocytes. These antigens react with specific antibody to form a sensitized lymphocyte which then reacts with rabbit complement. This results in the formation of holes in the cell membrane of those lymphocytes with attached antibody, allowing a dye to pass into the lymphocytes that have been killed. Thus the dark cells seen via the inverted phase microscope are dead cells containing dye. In a typical positive well, all the cells will be dead. The antisera used for these reactions are derived from multiparous women.

How are these antigens controlled genetically? The genes for HL-A antigens are located on an autosomal chromosome, with each child receiving one chromosome from his mother and one chromosome from his father. Two loci controlling a different series of antigens are located on each chromosome. Therefore, there are two-to-four antigenic possibilities for each offspring. An individual with only two HL-A antigens is rare, but this could occur if he received an identical pair from his mother and father. More common but still unusual and occurring in 10% of the population is the presence of only three HL-A antigens; the majority of people have four HL-A antigens. One should distinguish between the incidence of the antigens on a given chromosome, or gene frequency, and phenotypic frequency which is the incidence actually observed in people. The gene frequency of a given antigen is approximately one half the phenotype frequency. Because they are inherited in a Mendelian dominant fashion, there are four types of offspring with respect to HL-A antigens that a pair of parents may have (Fig. 1). Therefore, 25% of siblings will be HL-A identical. Also every child will share one haplotype, which is one pair of antigens controlled by the two genes on one chromosome with each parent. Therefore, a child will share two of the antigens of his father and he will usually have two additional antigens from his mother which are different from those of his father. There is also the chance, which is less than 1:4, that a sibling will share no HL-A antigens with another sibling.

What about the nomenclature of these antigens? HL-A designates antigens recognized by the World Health Organization Committee, which set up criteria for their identification. To be recognized, there must be two monospecific antisera which define the HL-A antigen and which do not overlap with other recognizable HL-A antigens. Tentative recognition is given to an antigen, when one monospecific serum identifies it and many labs recognize it. This is termed the W-classification system and what was orginally designated W-27 is now designated HL-A 27. HL-A antigens have frequently been discovered in a number of labs simultaneously, which has led to confusion because they are given local designations. For example, HL-A 5 has been designated by local groups as AO 45, BT 25, 4C, TO 5, DA 5, and TE 11. Obviously, it is easier to speak of HL-A 5, so order is gradually coming into the field.

Where do we stand in discovering these antigens? In the first segregate series controlled by one genetic locus, the gene frequency of those ten W and HL-A antigens already discovered adds up to 0.978. If all antigens have been discovered, the gene frequencies should add up to 1.00. On the second segregate series, 15 W and HL-A antigens have been discovered, but their gene frequency adds up to only 0.897, so approximately 10% of this group of HL-A antigens remain undiscovered. There is a difference in the racial distribution of these antigens. For example, HL-A 2 is found in 50% of Caucasians while HL-A 9 is found in over 70% of Orientals. One can see that a difference of gene frequency of these HL-A antigens would provide a basis for difference of disease incidence in different racial groups which

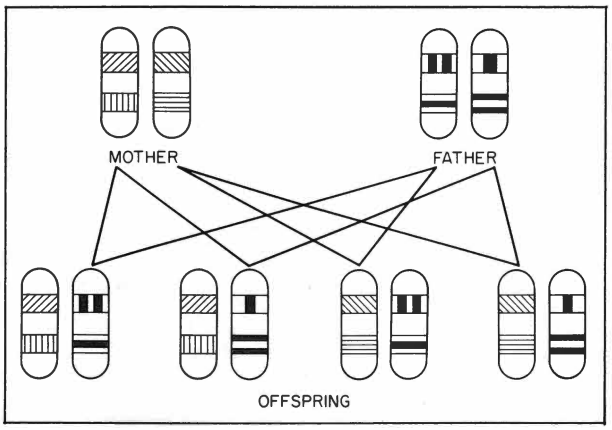

Fig. 1—This illustration depicts a pair of autosomal chromosomes from each parent with the two HL-A loci on each chromosome and the pattern of inheritance of the HL-A antigens; 25% of the siblings will be HL-A identical.

is relevant to our patient today. The association of HL-A 27 with ankylosing spondylitis and with Reiter's syndrome is striking.

Caution should be introduced, however, about the association of an HL-A antigen and a disease state. For example, some investigators have found a significant association of HL-A 5 with Hodgkin's disease while others have found no significant association at all. This is not very surprising, because if 21 antigens are studied for association with a disease, the chances are that one antigen will be significantly associated with the disease by chance if the level of significance is $P = 0.05$. Therefore, a correction factor is introduced. The level of significance is divided by the number of antigens studied in the search for an association. In the case of 21 antigens, the P value would have to be less than 0.0024 to have a significant association.

Two other points should be made about this system of antigens—one is that HL-A antigens are found on the surface of all somatic or nucleated cells, so they are accessible to involvement with diseases; secondly, this is the most polymorphic genetic system found so far in man, so it lends itself to individualization. Even though the incidence of a disease may be very small, there is a real possibility that it will be found associated with a given HL-A antigen.

Dr. Irby: Although the possible association of HL-A antigens with disease susceptibility has been investigated for many years, it has been only within the past two years that three convincing examples have come to light (Table 1). In psoriasis, there is an increased disease incidence in first degree relatives and in adult celiac disease, the incidence in relatives is six times normal. Patients with rheumatic diseases have provided many interesting and significant correlations with HL-A antigens (Table 2). Earlier studies and subsequent HL-A antigen investigators have demonstrated a striking relationship between HL-A 27 and ankylosing spondylitis and between HL-A 27 and Reiter's syndrome (Tables 3, 4).

Dr. Macdonald: Some years ago the principle way of classifying uveitis was on the basis of granulomatous uveitis, in which the primary inflammatory cell was the epitheloid cell. In the anterior segment of the eye, the exudation was that of a heavy proteinaceous material with many synechiae, compared to the nongranulomatous variety, in which the cell type was nonepitheloid with generally a much less severe reaction. In the granulomatous types of uveitis, or in iridocyclitis, the actual offending agent was considered to be present. For some years, this classification was expounded by Dr. Woods at Johns Hopkins University. At present, uveitis is generally classified according to where it is found in the eye, that is, anterior uveitis (involving mainly the iris), both segments (involving the iris, ciliary body, and choroid), and those involving only the choroid. It is of particular interest that the leading cause of anterior segment disease is unknown; in both segments, the third most common cause is unknown; and in the posterior or choroidal variety, many are unknown. Much work remains in order to properly determine the basic etiologies of uveitis. In the anterior segment variety, ankylosing spondylitis appears to be the third most common cause in this series.

In the literature, a system for studying the problem of sympathetic ophthalmia was propounded by Elschnig in 1910. He proposed that melanins in the uveal pigment of the eye where indeed the exciting factors. The studies were carried out by complement fixation tests. This has subsequently raised the question of the role of melanin in the eye. A further interesting question of the relationship of pigmentation in the skin to the Vogt-Kayanagi-Harada syndrome of vitiligo, poliosis, and dysacousis has been raised.

As we progress through clinical and research experiences, our concepts necessarily change. In 1941 Dr. Woods at Johns Hopkins reported a study of 343 cases of uveitis of which 80% were caused

TABLE 1

HL-A ASSOCIATED WITH DISEASE*

Celiac Disease	HL-A1	$2\frac{1}{2}$–3 × Normal	Stokes, 1972
	HL-A8	3 × Normal	Falchuk, 1972
Ch. Active Hepatitis	HL-A1		
	HL-A8	3 × Normal	Mackay, 1972
Psoriasis	HL-A17		Russell, 1972
	HL-A13	3 × Normal	White, 1973
Ankylosing Spondylitis	HL-A27	10 × Normal	Schlosstein, 1973 Brewerton, 1973

Also some correlation lymphoma, multiple myeloma, SLE, and lymphoblastic leukemia with various phenotypes HL-A.

* The association of certain HL-A antigens with disease states has proven to be more than a chance correlation.

by tuberculosis. In 1960 Dr. Woods reported another series including 134 cases of uveitis. In this study, only 20% of the cases were caused by tuberculosis, whereas 36% were caused by toxoplasmosis, 13% by histoplasmosis and 22% were of unknown etiology. Dr. Kaiser reported a study involving 110 patients with uveitis, and 38% of these were caused by histoplasmosis which was the largest single group. Much of this is, of course, attributable to the fact that Louisville lies in the Ohio Valley which is endemic for *Histoplasma capsulatum*. In our series of over 400 cases of uveitis, 60% have been posterior (choroid), 18% anterior, 12% midsegment, and 10% involving all segments of the eye. Approximately 58% of the cases of anterior uveitis had an unknown etiology. There are probably two reasons for this. First, there are many types of diseases which will produce a purely anterior uveitis. Secondly, ophthalmologists are divided into two groups—one group does everything diagnostically; the second group treats them all with steroids and a large majority get well anyway. Therefore, many patients with the anterior uveitis appear to have no known cause. In our group with anterior uveitis, there were four cases of ulcerative colitis with spondylitis (6%) and six cases of Reiter's disease or other

TABLE 2

HL-A 27 IN RHEUMATIC DISEASE PATIENTS*

Condition	Presence of HL-A 27	Percentage	Source
Normal	119/1456	8%	Russell, 1972 Brewerton, 1973 Schlosstein, 1973 White, 1972
Rheumatoid arthritis	10/119	8%	Schlosstein, 1973
Gout	6/66	9%	Schlosstein, 1973
Ankylosing spondylitis	35/40	88%	Schlosstein, 1973
	72/75	96%	Brewerton, 1973
Reiter's syndrome	25/33	76%	Brewerton, 1973
Acute anterior uveitis	26/50	52%	Brewerton, 1973
Psoriasis	9/156	6%	White, 1972
	6/44	14%	Russell, 1972
Psoriasis/spondylitis	10/14	71%	Metzger, 1974

* A compilation of rheumatic diseases and HL-A 27 shows its strong association with ankylosing spondylitis, Reiter's syndrome, and psoriatic spondylitis.

TABLE 3

RACIAL ASPECTS OF ANKYLOSING SPONDYLITIS*

1. Random study McGuire VA H: 26 white, 3 black.—Toone 1949
2. Combined VA H study, 301 pts. 10% black.—Baum 1971
3. HL-A 27 absent in Black Africans.—Dausset
4. HL-A 27 only 4% in Black Americans.
5. HL-A 27 present in 8 of 10 Black American spondylitics.

* The above figures demonstrate the relationship of HL-A 27 and ankylosing spondylitis on Black Americans.

rheumatic diseases (8%). Therefore, 14% of our cases of anterior uveitis had some rheumatological problem. Ninety-six percent of the cases in our series with midsegment disease had no apparent diagnosis after workup. Two percent were diagnosed as spondylitis. Perhaps, a reason for the small number of diagnoses is that ankylosing spondylitis and sacroiliitis have, in the past, been overlooked.

Dr. Irby: So what can we conclude from this discussion?

1. There is an 88–95% incidence of HL-A 27 antigen in patients with ankylosing spondylitis and a 76% incidence in patients with Reiter's disease against an 8% incidence in a controlled Caucasian population.

2. First degree relatives of spondylitics have 20–30 times the risk of developing spondylitis and 50% of these will have HL-A 27.

3. The HL-A 27 antigen may be a useful tool in diagnosis or prognosis, particularly in early rheumatic disease patients including those with juvenile rheumatoid arthritis.

4. There is a possibility that HL-A 27 may predispose a patient to develop Reiter's disease or ankylosing spondylitis; however, much work needs to be done in this regard to implicate HL-A 27 as an etiological factor.

TABLE 4

HEREDITARY ASPECTS OF ANKYLOSING SPONDYLITIS*

1. 30× more prevalent among relatives of spondylitic patients than controls.—Stecher
2. 22.6× more prevalent in relatives of spondylitics than controls.—De Blecourt
3. W-27 found in 31/60 1° relatives.—Brewerton
4. No blood group association.

* The hereditary aspects of ankylosing spondylitis are clinically apparent as well as genetically significant.

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