Different HLA-D Associations in Adult and Juvenile Rheumatoid Arthritis

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ABSTRACT HLA-D typing was performed in 126 patients with juvenile rheumatoid arthritis. HLA-DW4, the antigen found in previous studies to characterize adult rheumatoid arthritis, had a significantly lower frequency in children with arthritis than in normal controls ($P < 0.04$). By contrast, in children the antigens HLA-DW7 ($P < 0.03$) and HLA-DW8 ($P < 0.01$) were increased compared to controls. The antigen TMo, detected with homozygous typing cells from a child with juvenile rheumatoid arthritis, was found to be related to the cross-reactive specificities HLA-DW7 and DW11. 46% of the patients with persistent pauciarticular arthritis of childhood typed for the antigen TMo, compared to only 1% of normal controls. Thus the relative risk for persistent pauciarticular arthritis in relation to the presence of TMo was 67.7 ($P < 0.0001$). These results provide evidence of fundamental differences between adult rheumatoid arthritis and arthritis of childhood. The latter group appears to include a population distinguishable clinically and characterized in these studies by the HLA-D determinant TMo.

INTRODUCTION

Arthritis in children often presents with manifestations that would be considered unusual in adults such as daily high fever, hepatosplenomegaly, and rash. Yet juvenile rheumatoid arthritis (JRA),[1] like lupus, polymyositis, or scleroderma with onset in childhood, has commonly been considered to be essentially the same disease as seen in the adult but modified by the younger age of the host. In recent years pediatric rheumatologists have attempted to classify JRA into several distinct onset syndromes (1). These subdivisions are based on the number of affected joints, pauciarticular or polyarticular, and on the presence or absence of daily intermittent fever, which defines the systemic form. Certain children with chronic arthritis may develop a clinical syndrome with spine and sacroilliac involvement similar to ankylosing spondylitis (2).

In previous publications from this laboratory (3) it has been reported that HLA-DW4, and the associated Ia-like antigen DRW4, are strongly associated with rheumatoid arthritis (RA) in Caucasian adult patients. The relative risk for development of RA was six times greater in HLA-DW4-positive individuals than in those who lacked the antigen (3). These results suggested that immunogenetic factors were involved in the development of RA of adult onset.

We have now investigated 126 Caucasian patients with JRA who were tested for HLA-A, B, and D antigens. The results are surprisingly different from what was found in adults. In children who met the criteria for JRA (1) the frequency of HLA-DW4 was lower than in controls whereas other antigens of the HLA-D locus were increased. The persistent pauciarticular group appeared to have a strong association with a new HLA-D determinant that was characterized with a typing cell from a child with JRA. This new antigen will be described.

METHODS

The 126 patients with JRA, entered at random in this study, were attending the pediatric rheumatology clinic, Texas Scottish Rite Hospital for Crippled Children, or were hospitalized at the Children's Medical Center, Dallas, Tex. They were under the care of one of us (C. W. Fink) and were classified on the basis of clinical criteria without knowledge of the HLA-typing results.

Criteria used for classification of patients were adapted from those previously published by the JRA Criteria Subcommittee (1). 67 patients had a pauciarticular onset, in 31 the onset was polyarticular, and in 28 it was systemic. The pauciarticular group was further subdivided into four subsets. Of the 67 patients with pauciarticular onset, 12 had had a chronic iritis and 10 were boys whose illness began after the age of 9 yr. Of the remaining 45 patients, 26 had a persistent pauciarticular course and 19 converted to a polyarticular form

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[1] *Abbreviations used in this paper*: DNV, double normalized values; JRA, juvenile rheumatoid arthritis; RA, rheumatoid arthritis.

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TABLE I
Clinical Classification of Patients with Juvenile Arthritis

Clinical group		Number
Pauciarticular onset		67
With iritis	12	
Males, after 9 yr	10	
Persistent pauciarticular	26	
Conversion to polyarticular	19	
Polyarticular onset		31
Systemic onset		28
Total		126

of the disease by later having a total of more than four involved joints (Table I).

Typing for the HLA-D locus determinants was performed by mixed lymphocyte culture with a microtiter procedure as previously described (4). Each reaction mixture contained 10^5 frozen and thawed responding lymphocytes and a similar number of frozen and thawed stimulating lymphocytes which had been previously irradiated (3,000 rad). The results obtained after scintillation counting were recorded on paper tape for input into a DEC System 10 computer (Digital Equipment Corp., Marlboro, Mass.). Analysis was performed by a program that printed the median values of triplicate counts and calculated the relative responses. Because of the variability in the capacity of frozen lymphocytes to perform both as responding and as stimulating cells it is customary in mixed lymphocyte-culture typing experiments to normalize the raw results. In the present experiments relative responses were obtained by the 75th percentile method, with horizontal (responder-wise) and vertical (stimulator-wise) stabilization (5, 6). The double normalized values (DNV) thus obtained have been shown to give better day-to-day reproducibility than other data reduction methods previously used. DNV were used for scoring the reactions as positive or negative. Responses to homozygous stimulators by responders with the antigen, usually gave DNV below 25%. Other responders known to have the antigen, however, produced DNV in the 26–40% range. Such values were coded as positive only if confirmed in a second culture. All subjects were tested on at least two occasions. Many were tested four–six times. The typing cells used were obtained locally or through exchanges with other investigators. They defined the 6th Workshop specificities DW1 through DW8 and the recently recognized (7th Workshop) specificity DW11 (Table II). In addition, cells from the JRA patient TMo, which appear to characterize a new specificity, related to the DW7-DW11 cross-reactive group (*vide infra*) were also used. The 7th Workshop homozygous typing cells 7W506, 7W534, 7W542, 7W566, 7W537, 7W535, and 7W527, used in the experiment given in Table V, are described in detail in the joint report of the 7th International Histocompatibility Workshop (7).

Typing for the HLA-D-related (DR) specificities was performed by cytotoxicity with isolated bone marrow-derived (B) lymphocytes obtained from peripheral blood by removal of thymus-derived (T) lymphocytes with neuraminidase-treated sheep erythrocytes. Ficoll-Hypaque-separated mononuclear cells (Ficoll, Pharmacia Fine Chemicals Inc., Piscataway, N. J.; Hypaque, Winthrop Laboratories, Sterling Drug Co., New York) were mixed with an equal volume of 1% neuraminidase-treated sheep erythrocytes (8), incubated at 37°C for 15 min, centrifuged at 200 *g* for 3 min, and incubated at 4°C overnight. Resuspended cell pellets were then underlayered with Ficoll-Hypaque solution and centrifuged at 400 *g* for 30 min. The purified B lymphocytes, recovered from above the second Ficoll-Hypaque, usually contained less than 5% rosette-forming cells and 70–90% were killed by a polyvalent rabbit anti-human Ia serum. The polyvalent xenogeneic anti-Ia serum was a serum raised in rabbits by immunization with purified Ia antigens from a lymphoblastoid B-cell line kindly supplied by Dr. Walter Bodmer. It was known to react with purified B lymphocytes, monocytes, and lymphoblastoid B cells including Daudi, but not with most peripheral blood lymphocytes, lymphoblastoid T cells, or fibroblasts (9). The cytotoxicity tests with B lymphocytes were incubated for 1 h with antiserum and for 2 h with a rabbit complement, both periods at 20°C. The sera used for defining DR specificities were of local origin and obtained through exchange with other investigators. They allowed definition of the 7th Workshop specificities DRW1 through 7 and WIa8.

RESULTS

The frequency of HLA-A, and B antigens. The distribution of HLA-A, and B antigens in 95 patients

TABLE II
Mixed Lymphocyte Culture Typing Cells Used in these Experiments

Specificity	Name	Number	Origin	Specificity	Name	Number	Origin
DW1	MDe	—	Local	DW4	JWo	Ref. lab.	Local
DW1	MLAU	Ref. lab.*	Navy‡	DW5	Cos	7W531	SF‡
DW2	LCh	—	Local	DW6	JGr	—	Local
DW2	TRAL	—	Navy	DW7	PBUR	7W534	Hsu‡
DW3	GS	Ref. lab.	Local	DW8	BCo	—	Local
DW3	JGi	—	Local	DW11	JLe	—	Local
DW4	AC	7W506	Local	DW11	KA	—	Local

* Ref. lab., not in workshop, but certified by the reference laboratory for the HLA-D specificity; other local homozygous typing cells were characterized with 6th and(or) 7th Workshop typing cells.

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with JRA was compared to that in 91 normal Caucasian controls. There were no statistically significant differences between the two groups. The frequency of HLA-B27 was 15% in the patients compared to 9% in the controls. Interestingly, in the group of boys with a pauciarticular onset after the age of 9 yr (group A2) 8 of the 10 patients were HLA-B27 positive ($P < 0.00001$). The relative risk for this type of disease estimated from the present results is 44.0, $P < 0.00002$.

The distribution of HLA-D antigens. The results of HLA-D typing are shown in Table III. The number of subjects typed for each specificity varied because of limited availability of some typing cells at various times in the course of the study. The most surprising finding is the low frequency of DW4 in JRA patients. It was found in only 9% of the 122 JRA patients compared to 20% of the normal controls. This was contrary to expectation, because DW4 was markedly increased in patients with adult RA. The antigens DW7, DW8, and TMo were more frequent in JRA patients compared to normal controls (Table III).

In Table IV, the patients have been divided into the three major clinical groups according to mode of onset. The lowest frequency of DW4 was observed in the pauciarticular group in which the elevations in DW7, DW8, and TMo were found.

The antigen HLA-DW7 was also found more frequently in patients with a systemic onset. This result however, is based on typing of only 14 patients and must be investigated further.

TMo, an HLA-D specificity that belongs to the DW7–DW11 cross-reactive group. TMo was discovered in random checkerboard cultures with cells from patients with JRA. TMo lymphocytes typed HLA-A2, AW26, BW15, BW37. The family checkerboard culture indicated that TMo inherited an A2, BW37, DRW7 haplotype from her father and an AW26, BW15, DRW- from her mother. Both haplotypes carry the same lymphocyte-defined determinant. Lymphocytes from either parent were stimulated very little by TMo cells (4 and 23%, respectively), but TMo responded to both parents at the one haplotype level (68% and 49%). Experiments performed to characterize the determinant TMo in relation to the known HLA-D specificities revealed that there was a relationship to DW7 and DW11. In repeated experiments TMo was not stimulated by typing cell 12-001, one of the two homozygous typing cells that defined DW7 in the 6th Workshop. Results of a checkerboard culture with selected 7th Workshop typing cells (7) are given in Table V. Cell AC, which served to characterize DW4 in both the 6th (2-005) and the 7th Workshops (7W506), was included as a control to show that DW7, DW11, and TMo are distinct from DW4. TMo as a responder was not stimulated by cells 7W534, 7W537, 7W527, and very little by JLe. In the reciprocal cultures, the same cells (except 7W534) gave low responses against TMo. On the other hand, strong stimulation was observed with cells 7W542, 7W566, and 7W535 with TMo as a responder. To further elucidate the relationship between TMo, DW7, and DW11, lymphocytes from 209 individuals, 139 normal panel members, and 70 JRA patients, were used as responders in typing experiments for the three specificities (Fig. 1). The results showed that both in normal individuals and in JRA patients there was overlap in the typing responses to the three cells used to define these specificities. In some individuals positive reactions to JLe (DW11) and 7W534 (DW7) occurred simultaneously. Because such people usually had, in addition, another defined HLA-D antigen, it seems likely that the overlapping specificities were caused by products of the same haplotype. Similarly, typing responses to TMo occurred either alone or in association with typing responses to the other two cells.

TABLE III
Frequency of HLA-D Antigens in Patients with JRA and Controls

Antigen	JRA patients		Normal controls		
	Number	Percent	Number	Percent	*P**
DW1	16/61	26	15/77	19	NS
DW2	13/87	15	24/84	29	NS
DW3	15/97	15	15/84	18	NS
DW4	11/122	9	17/84	20	<0.04
DW5	3/54	6	4/76	5	NS
DW6	11/96	11	8/79	10	NS
DW7	19/62	31	10/75	13	<0.03
DW8	25/98	26	7/82	9	<0.01
DW11	13/123	11	10/80	13	NS
TMo	25/123	20	1/80	1	<0.0005

* *P* values were obtained from the chi square test with Yates' correction.

Relative risk for various clinical forms of JRA with the HLA-D specificity, TMo. In view of the clinical heterogeneity of juvenile arthritis, the relative risks for each of the clinical groups, in relation to the presence TMo, were calculated separately. From the data given in Table VI, it can be seen that the strongest association was with the persistent pauciarticular group (group A3). The results indicate that a child having the antigen TMo has a 68-fold greater chance of developing this form of arthritis. Thus it would appear that the association between TMo and JRA with a pauciarticular onset, is largely a result of patients with the persistent pauciarticular form of the disease. In groups A1 (with iritis), B (polyarticular onset), and C (systemic onset), the relative risks were not significantly different from unity.

TABLE IV

Frequency of HLA-D Antigens in JRA Patients Classified According to Clinical Onset

Subjects	No.*	Frequency of HLA-D antigens									
		DW1	DW2	DW3	DW4	DW5	DW6	DW7	DW8	DW11	TMo
		%									
Normal controls	84	19	29	18	20	5	10	13	9	13	1
Onset of JRA:											
Pauciarticular	67	37	17	11	5§	9	17	32‡	37‖	13	28‖
Polyarticular	31	19	5‡	23	16	0	12	18	16	0	10
Systemic	28	13	21	16	11	7	0	43‡	13	18	14
All JRA	126	26	15	15	9‡	5	11	31‡	26§	11	20‖

* The actual number tested for each HLA-D antigen was, in most cases, less than the total number of patients studied.

‡ $P < 0.05$.

§ $P < 0.01$.

‖ $P < 0.001$.

Increased relative risks were observed also in groups A2 (males with onset after 9 yr of age) and A4 (converting to polyarticular) (Table VI).

Results of serologic typing for the HLA-D-related (DR) specificities. A small group of random JRA patients was typed for the HLA-DR specificities of the 7th International Histocompatibility Workshop by cytotoxicity with isolated B cells from their peripheral blood. The results of these experiments showed that HLA-DRW4 was not increased in JRA patients compared to controls; it was actually decreased in frequency (Table VII). The antigen WIa8 was elevated, resembling the distribution of the corresponding HLA-D antigens.

HLA-DW4 and DRW4 typing in patients with polyarticular JRA with positive-rheumatoid-factor tests. Eight children with polyarticular onset JRA and positive latex agglutination tests, most of whom also had subcutaneous nodules, were tested for HLA-DW4 and DRW4. Only one was found to be positive for HLA-

TABLE V

Results of Reciprocal Cultures between Homozygous Typing Cells Defining HLA-DW4, D7, DW11, and TMo

Responding cells	HLA-D	75th percentile*	Stimulating cells‡								
			506 AC	534 PBUR	542 JB12	566 EI	537 MA	535 JK	527 MPH	JLe	TMo
			DW4	DW7		DW11					
		cpm	*DNV %*								
7W534	W7	11610	104	04	08	89	65	68	41	20	118
7W542	W7	18666	113	10	01	78	91	63	82	112	49
7W566	W11	10319	135	33	NT	08	48	39	68	50	144
7W537	W11	19073	79	06	NT	01	01	01	24	11	31
7W535	W11	25252	98	23	NT	03	10	02	12	42	54
7W527	W11	7904	112	63	NT	NT	NT	NT	01	20	47
JLe	W11	15067	99	12	35	10	51	09	41	05	40
TMo		30970	75	09	56	63	10	100	07	35	07

* 75th percentile of values for each responding cell against all the stimulators expressed in counts per minute (cpm), which was used as 100% for the responder-wise (horizontal) stabilization of the data (5, 6).

‡ Cells identified by numbers used in 7th International Histocompatibility Workshop and local designations. Stimulating cells were x-irradiated with a dose of 3,000 rad.

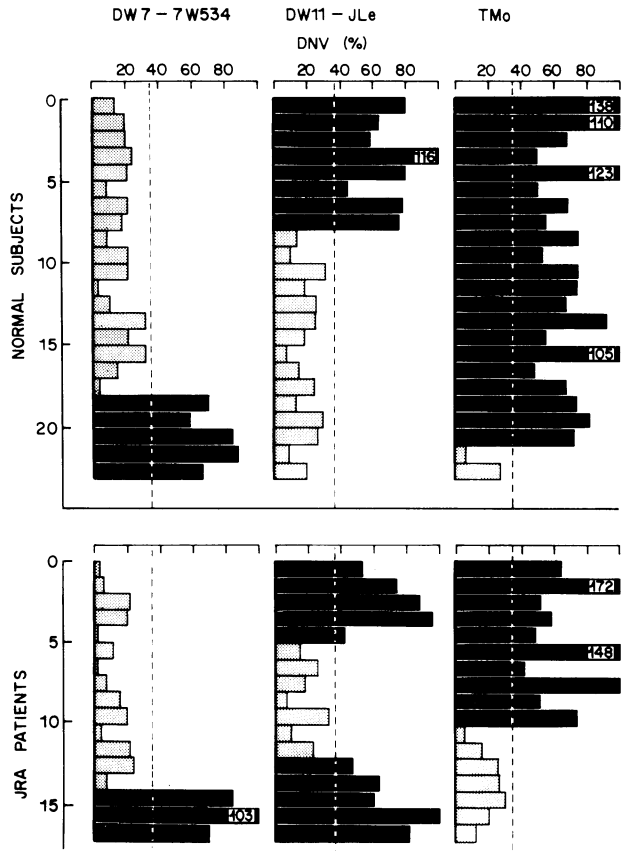

FIGURE 1 Relationship between HLA-DW7, DW11, and TMo. A panel of 139 normal subjects and 70 JRA patients were typed with cell 7W534 for DW7, cell JLe for DW11 and with cell TMo. Results are expressed as DNV. Typing reactions, shown by stippled bars, were below an arbitrarily chosen value of 35%. Many individuals in both groups typed for both DW7 and DW11. In the normal panel TMo was associated with DW11 in two persons. Among the JRA patients, TMo was partly associated with DW7 or DW11. Results for individuals who were negative with all three cells are not shown.

DRW4, this patient's arthritis began at 16 yr of age. It appears that the DW4-DRW4 antigens were not increased in this group of JRA patients, which most resemble the adult form of the disease.

DISCUSSION

In early experiments in this laboratory it was found that HLA-DW4 was increased in adult patients with RA compared to normal controls. Similar results were subsequently obtained in a second group of patients, confirming the association with several other HLA-DW4 typing cells. More recently, we have also found that the corresponding HLA-D-related (DRW4) serologic specificity was increased in Caucasian adults with RA (3). Both the elevated frequency of HLA-DW4 (10) and

TABLE VI
Relative Risk for Various Clinical Forms of Juvenile Arthritis with the Antigen TMo

Clinical classification	Number patients*	Percent positive	Relative risk	P‡
		%		
A. Pauciarticular onset	64	28	30.9	<0.001
1. With iritis	12	8	7.2	NS
2. Males, after 9 yr	9	20	22.6	<0.02
3. Persistent pauciarticular	26	46	67.7	<0.0001
4. Conversion to polyarticular	17	18	16.9	<0.02
B. Polyarticular onset	28	7	6.1	NS
C. Systemic onset	26	8	6.6	NS

* Normal Caucasian controls were 80, positive 1%.
‡ Relative risks and their significance were calculated according to Woolf (13).

that of DRW4 (11) in RA have been confirmed by other investigators. It was therefore surprising to find that children with RA only rarely had these antigens. In the present study, involving 126 JRA patients, the frequency of DW4 was not only significantly lower than in adult RA but was even significantly lower than in the Caucasian normal controls. HLA-DRW4, tested by cytotoxicity on isolated B cells, was also not increased in children with RA. Moreover, none of the clinically identified subgroups of JRA showed an increase in DW4. We then investigated a group of children with polyarthritis, rheumatoid factor, and subcutaneous nodules whose disease most closely resembles adult RA. Only eight such patients were available but, evidently, also in this group HLA-DW4/DRW4 was not

TABLE VII
Frequency of HLA-DR Antigens in Patients with Juvenile Arthritis and Normal Controls*

HLA DR	Percent positive: JRA $n = 29$	Percent positive: Controls $n = 73$	P
	%		
DRW1	10	21	NS
DRW2	24	26	NS
DRW3	17	27	NS
DRW4	3	32	<0.05
DRW5	10	6	NS
DRW6	0	9	NS
DRW7	10	15	NS
WIa8	26	8	<0.05

* HLA-DR antigens were identified by cytotoxicity with isolated B lymphocytes (see text).

increased because only one out of the eight was positive for the antigen.[2]

The decreased frequency of DW4 in JRA was an intriguing observation. Because it suggested that another HLA-D specificity might be increased, the typing experiments were extended to include the other known HLA-D specificities for which homozygous typing cells were available. The results showed increases in the antigens DW7, DW8, and TMo.

The classification of patients with juvenile arthritis, according to the pattern of disease during the initial 6 mo, appears to be a useful way of dividing these patients into clinical groups. It was interesting to observe that the lowest frequency of HLA-DW4 was in the children with a pauciarticular onset. It was also this group that most frequently had the antigens DW7, DW8, and TMo. The two observations are likely to be related. HLA-DW7 occurred also with a higher frequency in the systemic group, but because this result is based on the typing of only 14 patients, interpretation of its significance must await further studies.

The clinical classification we have used separated boys who began their disease after the age of 9 yr from the 67 children with a pauciarticular onset. This small group was strongly associated with HLA-B27. It has been observed that boys with late onset pauciarticular arthritis have many hallmarks of early ankylosing spondylitis (2). Several of our patients in this group had sacroiliitis suggesting a similar relationship.

TMo is a JRA patient. The fact that she is homozygous for the HLA-D locus was first discovered in checkerboard cultures among random JRA patients and subsequently confirmed by family study. To characterize the determinant involved, TMo was compared to other typing cells for all available specificities. The results of reciprocal cultures suggested that the determinant TMo is related to both DW7 and to the new specificity DW11 (previously called LD17). There was clearly no relationship to DW4 or any other of the HLA-D specificities tested. The results of parallel typing experiments revealed that some cells that typed positive for TMo, also gave typing reactions to DW7 and DW11. The relationship between the three determinants (Fig. 1) is reminiscent of similarly complex patterns of serologic reactions previously encountered in HLA. It should be remembered, however, that the majority of the TMo-positive individuals were JRA patients. Only two normal panel members were positive for TMo. One possible interpretation is that the results of mixed lymphocyte culture are governed by complex genetically determined structures. To obtain the TMo-typing response, some individuals would be required to possess, in addition to DW7 or DW11, or both, an extra determinant that characterizes TMo. In other persons TMo may exist alone and the determinants that characterize DW7 or DW11 are not detectable; this has thus far been observed only in JRA patients.

The presence of TMo was associated most strongly with the persistent pauciarticular form of arthritis. In this group, the frequency of the antigen TMo was 46%, compared to 1% in the normal controls. The relative risk was extremely high (67.7). Thus, it appears likely that the increased frequency of TMo in the pauciarticular onset group was largely because of its high frequency in patients with the persistent pauciarticular form of juvenile arthritis. Considering the imperfections of the clinical classification, it seems possible that almost all of the positive children belong in one group. The two patients who began their disease at an older age and the three children who developed a few more joints than the maximum of four used in the classification, may in reality belong to the same group. There were however some TMo-positive children in the systemic and the polyarticular onset groups. These patients will be followed to determine whether their course will differ from other patients with the same onset.

It is well known that the distribution of histocompatibility antigens varies greatly in subjects of different ethnic backgrounds. Care was therefore taken in the present study to limit the analysis to Caucasian patients living in and around the Dallas area. The two groups of patients with adult RA previously studied (3) were drawn from the same population. The difference in the HLA-D typing of adult and juvenile arthritis patients is therefore most likely because of a difference in the nature of the disease. The frequency of TMo in adult RA is currently being investigated. Preliminary results suggest that it may be somewhat higher than in normal controls, but the frequency is nothing like that of the pauciarticular group of JRA. Additional studies with typing cells for HLA-DW7 are also currently underway.

The mechanism of these and other HLA and disease associations is presently unknown. One possibility to be considered in RA, is that histocompatibility-linked control of the immune response may be involved. Such an effect could be exerted through specific immune-response genes, or it could be mediated through histocompatibility-linked control of cellular interactions between lymphocytes of different subsets or between lymphocytes and macrophages (12). The increased frequency of certain histocompatibility antigens in patients could signal the existence of a susceptibility factor. However, in a benign condition such as persistent pauciarticular arthritis of childhood, it could just

[2] Two additional patients with polyarticular disease and rheumatoid factor have been found to be DRW4 positive, bringing the frequency to 3 out of 10 (30%). Definitive conclusions about this group must await testing of further patients.

as well be a gene conferring relative resistance. Family studies of TMo-positive patients will be required to establish the frequency of homozygosity and its effect. The preliminary results thus far suggest that most of the patients are TMo heterozygous. Interestingly, TMo herself, who is homozygous, suffers from typical systemic-onset disease with spiking daily elevations of temperature. Thoughtful collaboration between the clinic and the laboratory promises to be fruitful in the furthering of our understanding of the genetic mechanisms in patients with this group of diseases.

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