Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 1233–1237, February 1990
Immunology

Heritable major histocompatibility complex class II-associated differences in production of tumor necrosis factor α: Relevance to genetic predisposition to systemic lupus erythematosus

(autoimmune disease/lupus nephritis/DR2, DQw1/human leukocyte antigen/restriction fragment length polymorphism)

CHAIM O. JACOB*, ZDENKA FRONEK*, GAIL D. LEWIS†, MAY KOO*, JOHN A. HANSEN‡, AND HUGH O. MCDEVITT*

*Departments of Microbiology and Immunology, and Medicine, Stanford University School of Medicine, Stanford, CA 94305-5402; ‡Fred Hutchinson Cancer Research Center, Seattle, WA 98104; and †Genentech Inc., South San Francisco, CA 94080

Contributed by Hugh O. McDevitt, December 4, 1989

ABSTRACT **We report on the production of tumor necrosis factor (TNF)-α and TNF-β by mitogen-activated peripheral blood lymphocytes or enriched monocyte subpopulations from human leukocyte antigen (HLA)-typed healthy subjects. The results indicate that HLA-DR2- and DQw1-positive donors frequently exhibit low production of TNF-α, whereas DR3- and DR4-positive subjects show high levels of TNF-α production. No correlation between TNF-α levels and *HLA-A*, *-B*, and *-C* genotype was found. The relevance of this quantitative polymorphism to the genetic predisposition to lupus nephritis in systemic lupus erythematosus (SLE) patients was investigated. DR2, DQw1-positive SLE patients show low levels of TNF-α inducibility; this genotype is also associated with an increased incidence of lupus nephritis. DR3-positive SLE patients, on the other hand, are not predisposed to nephritis, and these patients have high TNF-α production. DR4 haplotype is associated with high TNF-α inducibility and is negatively correlated with lupus nephritis. These data may help explain the strong association between HLA-DR2, DQw1 in SLE patients and their susceptibility to nephritis.**

Tumor necrosis factor (TNF)-α, initially recognized because of its cytotoxic and antitumor activities (1), has a broad spectrum of biological properties ranging from regulatory effects on various normal cell types to inhibitory effects on parasites and viruses (2).

Although predominantly produced by mononuclear phagocytes, TNF-α is also produced by B cells, T cells, and natural killer cells (3, 4). Another cytokine produced by activated lymphocytes and originally called lymphotoxin (5) shares many of the biological activities of TNF-α and has recently been named TNF-β (6).

Despite the ability of these cytokines to mediate a wide range of activities, their complete biological function is not clear. It is, however, quite apparent that TNF-α and TNF-β are part of a network of interactive signals that orchestrate inflammatory and immunological events (7).

Linkage analysis and pulsed-field gel electrophoresis studies (8–11) have shown that the genes for both human and mouse TNF-α and TNF-β are closely linked and located within the major histocompatibility complex (MHC).

A striking association between different MHC class II haplotypes and various diseases, most of them autoimmune in nature, has been well established (12). One of these disorders, systemic lupus erythematosus (SLE), is a complex autoimmune disease with female predominance and a large spectrum of manifestations, ranging from mild arthralgia to devastating attacks on one or many target tissues, including the kidney, central nervous system, blood vessels, and myocardium (13).

Population studies have shown an association between SLE and MHC class II DR2 and/or DR3 antigen alleles (14). Despite this association, the role of these molecules in SLE is not clear. It is even questionable whether the MHC genes are the predisposing genes to the disease rather than markers for other closely linked gene(s). Because the TNF genes are located within the MHC, the possibility of the involvement of an "abnormal" TNF gene in autoimmune disease development is an attractive hypothesis (10). Indeed, we have obtained data suggesting that TNF-α may be involved in the pathogenesis of lupus nephritis in (NZB × NZW) F_1 mice. A restriction fragment length polymorphism (RFLP) in the TNF-α gene correlates with reduced levels of TNF-α production by NZW mice, and replacement therapy with TNF-α induced a significant delay in the development of nephritis in these F_1 mice (15).

We now present data showing that TNF-α production levels in humans are MHC class II associated and are associated with nephritis development in SLE patients. These experiments together with our prior (NZB × NZW) F_1 studies suggest that TNF-α is involved in the genetic predisposition to develop SLE nephritis.

MATERIALS AND METHODS

Subjects. Normal, unrelated laboratory personnel and blood donors from Stanford University Medical Center and the Fred Hutchinson Cancer Center in Seattle were serologically typed for MHC class I and class II antigens with standard microlymphocytotoxity tests (16). The study included 72 healthy male donors and 29 healthy female donors.

One hundred and five SLE patients from the Stanford University Medical Center, University of California at Los Angeles, and University of California at San Francisco clinics who fulfilled the American Rheumatism Association criteria for the diagnosis of SLE (17) were entered into the study. Patients were diagnosed to have SLE nephritis when they had evidence of nephritis documented either by renal biopsy, proteinuria, hematuria, or renal failure not due to another independent etiology. Patients were considered to have SLE without nephritis when they had been diagnosed with SLE for at least 5 yr with no evidence of nephritis because the risk of developing renal disease is the greatest in the first 5 yr after diagnosis. The SLE patients were serologically typed for

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MHC, major histocompatibility complex; HLA, human leukocyte antigen; TNF, tumor necrosis factor; PBL(s), peripheral blood lymphocyte(s); SLE, systemic lupus erythematosus; RFLP, restriction fragment length polymorphism; IFN-γ, interferon γ; PMA, phorbol 12-myristate 13-acetate; PHA, phytohemagglutinin; RR, relative risk.

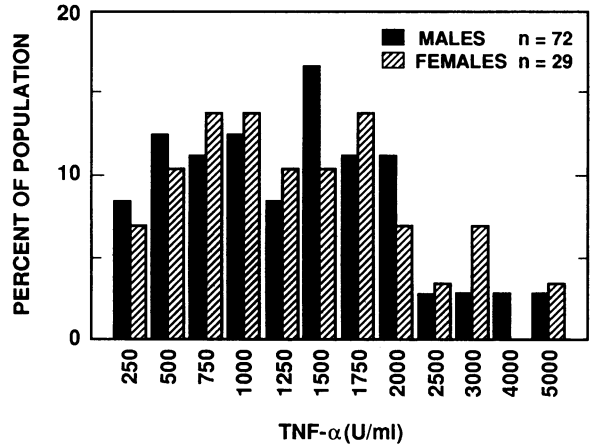

FIG. 1. Distribution of TNF-α production in normal healthy subjects. TNF-α levels were determined in 10^6 freshly prepared PBLs after stimulation with Con A (10 μg/ml) and PMA (2 ng/ml) as described. For each individual the mean value of three TNF-α determinations is given. SD is <10% for each subject. U, units.

HLA-DR and -DQ and genotyped for *DQα* and *DQβ* by using nucleotide sequence analysis (18).

Quantitation of TNF-α and TNF-β. Peripheral blood lymphocytes (PBLs) were separated by gradient centrifugation of heparinized venous blood on Ficoll/Hypaque. For preparation of peripheral blood monocytes, PBLs were incubated at 37°C for 45 min followed by vigorous pipetting and washing out of the nonadherent cells. PBLs (10^6 and 2×10^6 cells per ml) or enriched monocytes (10^5–5×10^5 cells per ml) were activated *in vitro* with a variety of mitogens including Con A, phytohemagglutinin (PHA), phorbol 12-myristate 13-acetate (PMA), lipopolysaccharide from *Escherichia coli*, and recombinant human interferon γ (IFN-γ) (Genentech) at different doses alone or in various combinations. Each experiment was done in triplicate. The cells were incubated for ≈60 hr at 37°C. Then the supernatants were harvested by centrifugation and stored at −20°C to be assayed at the same time.

TNF levels were assayed by using a bioassay that quantitates the TNF-induced cytotoxicity on L-M fibroblasts as described (19). TNF-α-specific or TNF-β-specific activities were determined in duplicates by incubation of every second tube of each duplicate with monoclonal anti-human TNF-α (enough to neutralize >5000 units of TNF-α per sample) at 4°C overnight before the cytotoxicity assay. The monoclonal anti-human TNF-α used is an IgG1 monoclonal antibody purified from ascites fluid with a titer of $>5 \times 10^5$ neutralizing units per ml (20).

About half of the samples were also tested by using a described specific ELISA (21). A good correlation was found when supernatants of stimulated PBLs were tested by both the bioassay and the ELISA (data not shown). Because the highest levels of TNF production were achieved when PBLs were activated by Con A at 10 μg/ml and PMA at 2 ng/ml, the data in the paper are given from this method of activation unless otherwise stated.

RFLP Analysis. DNA was extracted directly from PBLs or from Epstein–Barr virus-transformed PBLs. Ten micrograms of genomic DNA was digested with *Nco* I, according to the manufacturer's specifications. Electrophoresis, blotting, and hybridization were done using standard methods as described (15).

T-Cell Proliferation Assay. PBLs were activated with Con A (1–10 μg) or PHA (1–5 μg) for 2 or 3 days, and the proliferation capability was assessed by measuring tritiated thymidine ([^{3}H]dThd) incorporation as described (22).

Statistical Analysis. Fisher's exact test was used (23). In large-sample size groups, Woolf's formula for χ^2 was employed. Haldane's modification of Woolf's formula was used in samples containing zero (12). Relative risk (RR) calculations were done according to Woolf and Haldane (12).

RESULTS AND DISCUSSION

The production of TNF-α and TNF-β by activated PBLs and enriched monocytes of HLA-typed healthy donors was studied.

Because monocytes, T cells, B cells, and large granular lymphocytes can produce TNF-α, the response of the whole cell population may be more relevant biologically than that of enriched subpopulations (24). Moreover, the levels of TNF-α production by enriched monocytes correlated well with the production of this cytokine when total PBLs were used from the same person ($P = 0.01$ by linear regression analysis). Thus, to avoid further manipulation of cells *in vitro*, unsep-

Table 1. Comparison of TNF-α and TNF-β production between representative high-producer and low-producer age-matched Caucasian male donors

Source	Cells, no.	Inducing agents(s)*	Cytotoxic activity, units/ml			
			Donor A		Donor B	
			TNF-α	TNF-β	TNF-α	TNF-β
Serum			1	1	1	1
Unfractionated PBLs	10^6	None	7	2	8	5
Unfractionated PBLs	10^6	PMA (2 ng/ml)	1500	180	120	50
Unfractionated PBLs	10^6	Con A (1 μg/ml)	720	50	50	10
Unfractionated PBLs	10^6	Con A (10 μg/ml)	1600	120	150	12
Unfractionated PBLs	10^6	Con A + PMA	2200	240	200	105
Unfractionated PBLs	10^6	PHA	160	100	45	15
Unfractionated PBLs	10^6	PHA + PMA	1900	160	110	45
Unfractionated PBLs	10^6	LPS + PMA (5 ng/ml)	1550	210	120	50
Unfractionated PBLs	10^6	Con A + PMA + IFN-γ	2100	240	180	110
PBLs (adherent cells)	5×10^5	None	8	2	8	8
PBLs (adherent cells)	5×10^5	LPS + IFN-γ	290	8	45	7

Despite the significant differences in TNF-α production between these two subjects, their [^{3}H]dThd values in response to Con A (10 μg/ml) or PHA (1 μg/ml) are quite similar: 34,200 ± 1300 cpm and 21,500 ± 2000 cpm, respectively, for donor A and 32,800 ± 1800 cpm and 25,000 ± 1200 cpm, respectively, for donor B.

*Unless otherwise noted, given in the following concentrations: LPS (lipopolysaccharides), 10 μg/ml; IFN-γ, 100 units/ml; PMA, 2 ng/ml; Con A, 10 μg/ml; PHA, 10 μg/ml.

†Mean values of three experiments are given. SD is <10% in each of the columns.

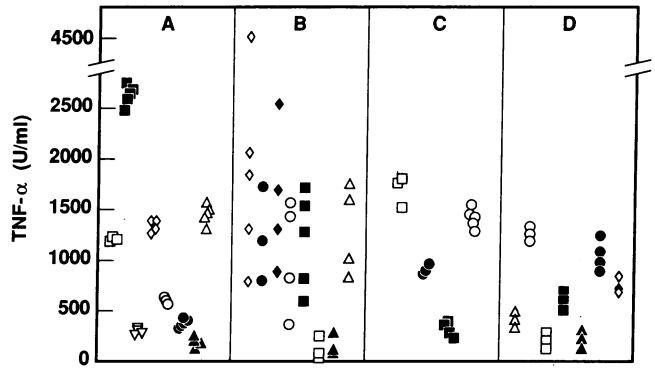

FIG. 2. Comparison of TNF-α production in randomly selected males (*A*), premenopausal females (*B*), postmenopausal women (*C*), and SLE female patients (*D*). Each symbol represents one subject tested 3–5 times at random intervals. U, units.

arated PBLs were used routinely for evaluation of TNF production.

Significant interindividual differences in the level of TNF-α inducibility were observed in normal healthy individuals (Fig. 1). Several lines of evidence support the notion of stable, interindividual differences in production of TNF-α rather than merely different overall sensitivities of PBLs from different donors to the particular stimulation conditions used.

(*i*) Various methods were used to induce TNF production *in vitro*. These included lipopolysaccharides, IFN-γ, Con A, PMA, and PHA at different doses and in various combinations. The results show that Con A plus PMA gave the highest level of inducibility of TNF-α and TNF-β. However, the interindividual differences between low producers and high producers are evident in all methods of activation and at different doses. Table 1 shows an example of such an experiment.

(*ii*) The levels of TNF-α production in the same subject were highly reproducible when males were tested randomly 3–5 times (Fig. 2*A*), suggesting that these interindividual differences are stable. In females, however, significant fluctuation of the levels of TNF-α could be demonstrated in randomly repeated tests (Fig. 2*B*). It is noteworthy that healthy females and males produce similar levels of TNF-α (Fig. 1), but the critical variance is in fluctuation with repeated testing in females. Because of these differences between males and females, the data shown in Tables 1 and 2 and Fig. 3 refer to male subjects only. This fluctuation in TNF-α level was greatly reduced in postmenopausal women tested repeatedly (Fig. 2*C*), suggesting that TNF-α production is regulated by sex hormones.

(*iii*) Proliferative capabilities of PBLs from different donors in response to the same mitogen used for TNF activation were tested. There was no correlation between the proliferative response to the mitogens and the capability to produce TNF-α in response to the same stimuli ($P > 0.05$, by linear regression analysis). Together these experiments imply that the differences in TNF-α levels are not due to individual differences in responsiveness to these mitogens or qualitative different kinetic responses to these mitogens but rather due to heritable interindividual variations.

Table 2. MHC class II distribution of TNF-α inducibility in normal donors (n = 72)

HLA class II allele*	Donors, no.	Individuals†, no. Low TNF	Intermediate TNF	High TNF
DR1	13	5	2	6
DR1 (non-DR2)	7	1	2	4
DR2	32	13	8	11
DR2 (non-DR3 or -4)	23	13	4	6
DR3	18	0	4	14
DR3 (non-DR2)	15	0	1	14
DR4	21	0	4	17
DR4 (non-DR2)	15	0	3	12
DR5	14	4	4	6
DR5 (non-DR2)	9	1	4	4
DR6	15	4	4	7
DR6 (non-DR2)	13	2	4	7
DR7	5	0	1	4
DR7 (non-DR2)	4	0	0	4
DR8	5	0	2	3
DR8 (non-DR2)	4	0	1	3

Note that the data were first analyzed by Wilcoxen rank-sum test, comparing the values for TNF-α in $DR2^+$ or $DR2^-$ individuals. The results of this analysis established that lower levels of TNF-α are associated with HLA-DR2 regardless of "cut-off" levels of TNF-α ($P < 0.01$).

*Established serologically.

†Low TNF, <500 units/ml; intermediate TNF, 500–1000 units/ml; high TNF, >1000 units/ml.

TNF-α and TNF-β were not detected in the serum of normal individuals, even in donors capable of producing very high levels of TNF-α by their activated PBLs *in vitro* (e.g., Table 1).

Under the experimental conditions used, a wide variation of TNF-α production, from a low of 20–30 units/ml to a high of 5000 units/ml, without any particular peak, was seen in normal healthy subjects. We have, therefore, arbitrarily divided TNF-α production into low production levels (0–500 units/ml), intermediate production levels (500–1000 units/ml), and high production levels (>1000 units/ml). Large ranges of levels were chosen for this subdivision to control for errors inherent from manipulations, cell counting, and assays. With this subdivision, a correlation between MHC class II genotype and the capability to produce TNF-α *in vitro*

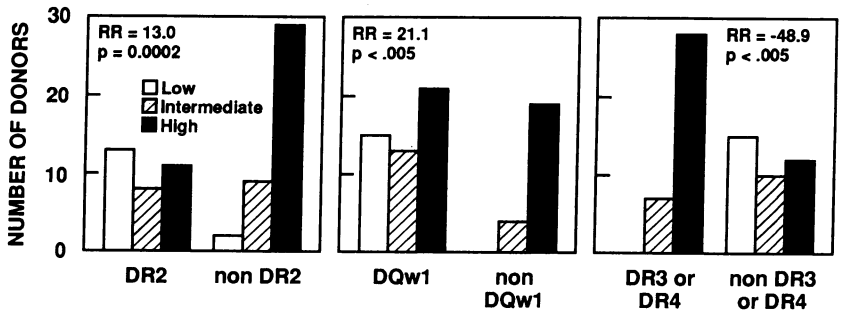

FIG. 3. Association of TNF-α inducibility with MHC class II in normal male donors (n = 72). HLA class II alleles were established serologically. RR is calculated as the comparison of having a specific MHC class II allele with low TNF-α (<500 units/ml) versus not having this allele and TNF-α >500 units/ml. For example, RR for DR2 is equal to [DR2] × {low TNF}/[non-DR2] × {high TNF}. P is calculated by using Fisher's exact two-tailed test or Haldane's modification of Woolf's formula for sets containing zero.

is demonstrated (Table 2 and Fig. 3). Thus, DR2- and DQw1-positive normal subjects are associated with low TNF-α production, whereas DR3- and DR4-positive individuals almost always show high levels of TNF-α production. As shown in Fig. 3 these results are highly significant. The RR for having low TNF-α production is 13 times higher in a DR2-positive individual than in a non-DR2 person. If the DQw1 specificity that includes DR1, DR2, and DRw6 is considered, the RR of low TNF-α production is 21 times higher in DQw1-positive subjects in comparison with non-DQw1-positive individuals. Conversely, DR3- or DR4-positive individuals have a RR for high TNF-α production of $\approx$49 compared with non-DR3 or -DR4 subjects.

Interestingly, no correlation was found between TNF-α production and MHC class I genotype, despite the fact that the TNF genes are located closer to MHC class I genes than to class II genes. Furthermore, there was no correlation between TNF-β production *in vitro* and MHC class I or class II genotype (data not shown). A RFLP, originally described by M. Steinmetz (personal communication), distinguishes DR2-positive individuals [10.5-kilobases (kb) *Nco* I fragment] from most DR3-positive individuals (5- and 5.5-kb bands). However, this RFLP pattern does not correlate with the quantitative functional polymorphism described here (e.g., Fig. 5).

This quantitative variation in TNF-α inducibility may be relevant to the MHC-associated genetic predisposition to SLE. Of the two haplotypes (DR2 and DR3) associated with SLE, only DR2, DQw1-positive SLE patients show an increased risk of developing lupus nephritis (Fig. 4 and ref. 18). These DR2, DQw1-positive SLE patients produce low levels of TNF-α (Fig. 4). On the other hand, DR4 is negatively associated with SLE nephritis (Fig. 4 and ref. 18). As with healthy individuals, among SLE patients the only group significantly associated with low TNF-α production was the DR2- and DQw1-positive group (Table 3). DR3- and/or DR4-positive SLE patients are actually negatively associated with low TNF production (RR = 0.15), just as are normal DR3 and DR4 subjects.

Because glucocorticoids have been shown to reduce both the mRNA and protein levels of TNF-α (25, 26), the fact that SLE patients have lower TNF-α production than healthy individuals may be accounted by the fact that many SLE patients are on steroid therapy. However, when the SLE patient population is divided into those on steroid therapy and those without such therapy, it is clear that the differences between the low and high production of TNF-α cannot be explained on the basis of this treatment alone (Table 3). On the other hand, development of lupus nephritis does correlate with low TNF-α production (RR = 4.3).

It is also interesting to note that TNF-α levels tend to fluctuate much less in SLE female patients than in healthy age-matched females (Fig. 2*D*).

Fig. 5 represents a multiplex SLE family. All three SLE patients in this family produce low levels of TNF-α, but they were also on steroid therapy. The nonaffected siblings are more informative, showing that the DR2-positive individuals produce low levels of TNF-α, whereas the DR3- or DR4-positive subjects are high producers of TNF-α. DR2/3 or DR2/4 heterozygotes show high levels of TNF-α production, suggesting a dominant effect of DR3 and DR4.

The data presented suggest that low TNF-α production may be involved in the genetic predisposition to lupus nephritis, similar to the (NZB $\times$ NZW) F_1 lupus nephritis model system (15) and may help explain the association between HLA-DR2/DQw1 and susceptibility to SLE nephritis. However, a substantial portion of SLE patients are DR3 associated; clearly these patients do not have low TNF-α production, similar to MRL-*lpr/lpr* and BXSB mice, which also show high levels of TNF-α production (C.O.J., G.D.L. and A. M. Stall, unpublished work). This result suggests that SLE is not a single condition but rather can be subdivided

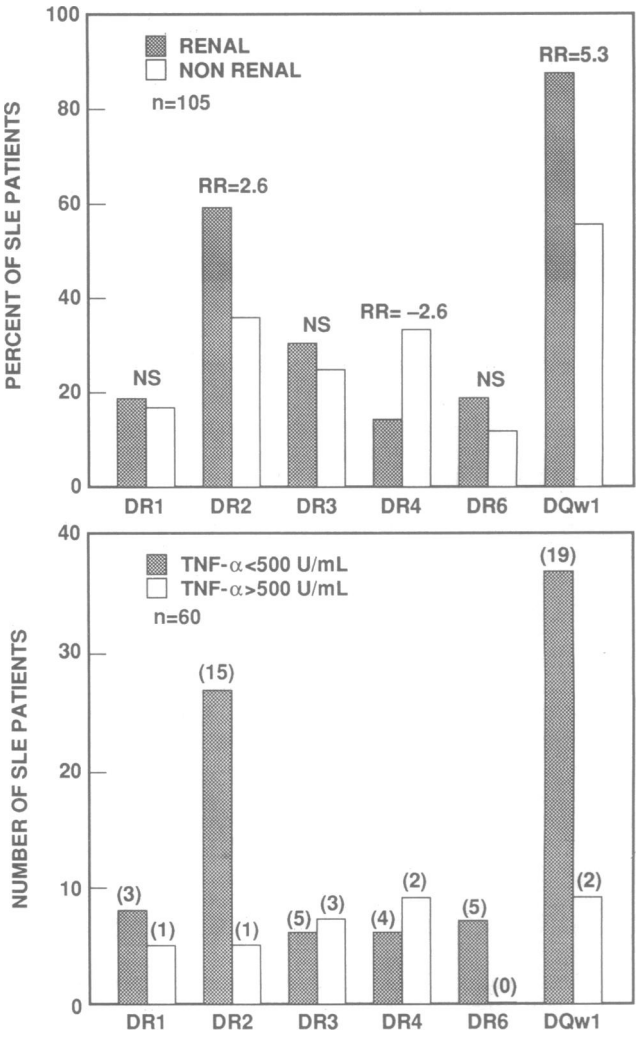

FIG. 4. MHC class II distribution and TNF-α production in SLE patients. HLA class II alleles were established serologically and by nucleotide sequence analysis for DQ haplotype alleles. RR of a particular class II allele and renal disease is calculated versus the same class II allele and non-renal disease. NS, not significant. The number of SLE patients with renal disease is given in parenthesis.

Table 3. MHC class II association and TNF-α inducibility in SLE patients

HLA class II allele*	TNF-α inducibility <500 units/ml (n = 41)	TNF-α inducibility >500 units/ml (n = 19)	RR†	P‡
DR2	27	5	5.4	0.004
Non-DR2	14	14		
DQw1	37	9	10.3	0.0005
Non-DQw1	4	10		
DR3 and/or DR4	12	14	0.15	0.001
Non-DR3, -4	29	5	(−6.77)	
SLE nephritis	22	4	4.3	0.01
Non-renal SLE	19	15		
Steroid use	28	10	NS	
No steorid	13	9		

NS, not significant.
*Established serologically.
†RR of class II allele and low TNF-α inducibility (<500 units/ml) versus another allele and TNF-α >500 units/ml.
‡P calculated using Fisher's exact two-tailed test.

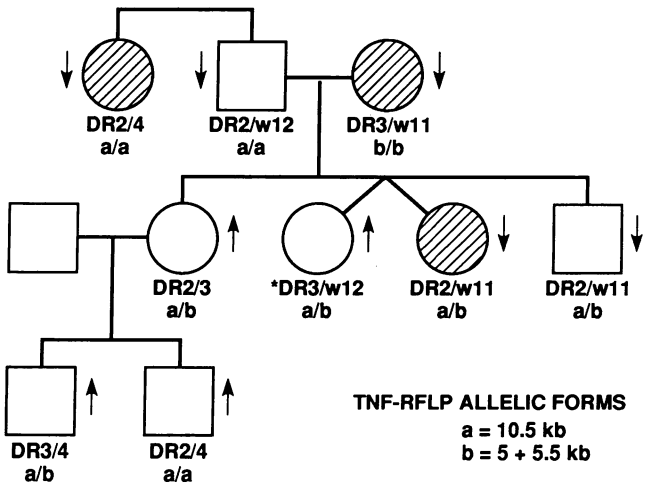

FIG. 5. Segregation of TNF-α production with MHC class II genotype in a multiplex SLE family. Shaded subjects have SLE. MHC class II alleles shown for each subject were determined serologically and by nucleotide sequence-specific analysis. *, Although this individual does not meet the American Rheumatism Association criteria for SLE, she has a positive antibodies to nuclear antigens test and an infant daughter with neonatal SLE who was not studied because of her age. The husband in the second generation was not studied. RFLP alleles for *Nco* I digestion are given as a, b, or ab, as identified in the inset. ↓, Levels of TNF-α <500 units/ml; ↑, levels of TNF-α >1000 units/ml.

into at least two subsets: one associated with DR2/DQw1, increased susceptibility to lupus nephritis, and low TNF-α production; the other with DR3 and high TNF-α production associated with lupus without nephritis.

We do not know which gene in the MHC is responsible for the association with low TNF-α production or whether the class II molecules are markers for another gene. Obviously linkage and mapping studies are necessary to answer this question. Alternatively, there could be a necessary functional association between MHC class II genotype, TNF-α levels, and susceptibility to SLE nephritis. Despite the fact that important questions remain open for further investigation, the data suggest that both TNF-α and MHC class II may be involved in the pathogenesis of lupus nephritis.

This work was supported by National Institutes of Health Grants to H.O.M. and J.A.H. C.O.J. is an Irvington Institute Fellow. Z.F. was supported by an Arthritis Foundation Postdoctoral Fellowship.

1. Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N. & Williamson, B. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3666–3700.
2. Beutler, B. & Cerami, A. (1987) *N. Engl. J. Med.* **316,** 379–385.
3. Sung, S. S., Bjorndahl, J. M., Wang, C. Y., Kao, H. T. & Fu, S. M. (1988) *J. Exp. Med.* **167,** 937–953.
4. Sung, S. S., Jung, L. K. L., Walters, J. A., Chen, W., Wang, C. Y. & Fu, S. M. (1988) *J. Exp. Med.* **168,** 1539–1551.
5. Ruddle, N. H. & Waksman, B. H. (1968) *J. Exp. Med.* **128,** 1267–1279.
6. Goeddel, D. V., Aggarwal, B. B., Gray, P. W., Leung, D. W. & Nedwin, G. E. (1986) *Cold Spring Harbor Symp. Quant. Biol.* **51,** 597–610.
7. Old, L. J. (1987) *Nature (London)* **326,** 330–331.
8. Nedwin, G. E., Naylor, S. L., Sakaguchi, A. Y., Smith, D., Jarrett-Nedwin, J., Pennica, D., Goeddel, D. V. & Gray, P. W. (1985) *Nucleic Acids Res.* **13,** 6361–6373.
9. Spies, T., Morton, C. C., Nedospasov, S. A., Fiers, W., Pious, D. & Strominger, J. L. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8699–8702.
10. Muller, U., Jongeneel, C. V., Nedospasov, S. A., Fischer-Lindahl, K. & Steinmetz, M. (1987) *Nature (London)* **325,** 265–267.
11. Dunham, I., Sargent, C. A., Trowsdale, J. & Duncan-Campbell, R. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7237–7241.
12. Tiwari, T. L. & Terasaki, P. I. (1985) in *HLA and Disease Associations* (Springer, New York), p. 19.
13. Steinberg, A. D. & Klinman, D. M. (1988) *Rheum. Dis. Clin. N. Am.* **14,** 25–41.
14. Woodrow, J. C. (1988) *J. Rheumatol.* **15,** 197–199.
15. Jacob, C. O. & McDevitt, H. O. (1988) *Nature (London)* **331,** 356–358.
16. Terasaki, P. I. (1965) in *Histocompatibility Testing* (Natl. Acad. Sci., Washington, DC), p. 171.
17. Tan, E. M., Cohen, A. S., Fries, J. F., Masi, A. T., McShane, D. J., Rothfield, N. F., Schaller, J. G., Talal, N. & Winchester, R. J. (1982) *Arthritis Rheum.* **25,** 1271–1277.
18. Fronek, Z., Timmerman, L. A., Alper, C. A., Hahn, B. H., Kalunian, K., Peterlin, M. B. & McDevitt, H. O. (1988) *Am. J. Med.* **85,** 42–44.
19. Mukavitz-Kramer, S. & Carver, M. E. (1986) *J. Immunol. Methods* **93,** 201–206.
20. Bringman, T. S. & Aggarwal, B. B. (1987) *Hybridoma* **6,** 489–507.
21. Prince, W. S., Harder, K. J., Saks, S., Reed, B. R., Chen, A. B. & Jones, A. J. S. (1987) *J. Pharmacol. Biomed. Anal.* **5,** 793–802.
22. Utsinger, P. D. & Young, W. J. (1977) *J. Clin. Invest.* **60,** 626–638.
23. Mathews, J. D. (1984) in *Detection of Immune Associated Genetic Markers of Human Disease*, eds. Simons, M. J. & Tait, B. D. (Churchill Livingstone, New York), p. 106.
24. Endres, S., Ghorbani, R., Kelley, V. E., Georgilis, K., Lonnemann, G., van der Meer, J. W. M., Cannon, J. G., Rogers, T. S., Klempner, M. S., Weber, P. C., Schaefer, E. J., Wolff, S. M. & Dinarello, C. A. (1989) *N. Engl. J. Med.* **320,** 265–271.
25. Beutler, B., Krochin, N., Milsark, I. W., Luedke, C. & Cerami, A. (1986) *Science* **232,** 977–980.
26. Waage, A. & Bakke, O. (1988) *Immunology* **63,** 299–305.