Association between a T Cell Receptor Restriction Fragment Length Polymorphism and Systemic Lupus Erythematosus

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Abstract

The present study was designed to test the possibility that T cell receptor genes are associated/linked to those involved in systemic lupus erythematosus (SLE). Genomic DNA was isolated from 31 unrelated Caucasian SLE patients, 34 unrelated Caucasian normals, 5 multiplex American Caucasian SLE families, 9 multiplex Mexican SLE families, and 13 unrelated Mexican normals. The DNA was digested with Pst I, electrophoresed, and transferred to membranes by the Southern blot method. The blots were probed with a cDNA probe for the alpha chain of the T cell receptor. 13 polymorphic RFLP patterns were recognized. 1.3- and 3.0-kb band pairs were observed in 15 of 31 of American Caucasian patients and 4 of 34 American Caucasian controls (chi square, 8.81; $P < 0.002$; relative risk, 7); there was no association of any RFLP pattern with Mexican SLE. The cDNA probe was cut with Rsa I, EcoR I, and Ava II into fragments corresponding to the V, J, C, and 3'UT regions. Only the fragment corresponding to the constant region reacted with the 1.3/3.0-kb band pair. These observations suggest that a genetic marker of the constant region of the alpha chain of the T cell receptor is associated with genes involved in SLE. (*J. Clin. Invest.* 1990. 86:1961–1967.) Key words: T cell receptor • systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE)[1] is a complex autoimmune disease due, apparently, to the combination of a number of factors. Hormonal, environmental, including perhaps infectious elements appear to act on top of a background of immune abnormalities. The immune abnormalities appear to result from defects in regulatory interactions between monocytes, T cells, and B cells. This leads to a loss of self tolerance and a hyperactive B cell system leading to the production of autoantibodies. An additional part of the background of SLE are genetic factors. The significant concordance (27–57%) for SLE in identical twins (1, 2), and the increased frequency of SLE (5–12%) and related immune abnormalities in nonidentical twins and in relatives of SLE patients suggest a genetic influence on the development of SLE (1, 3–9). Based on these observations, Winchester (10) has proposed a four-gene model for SLE, with variable penetrance. These putative genes may be linked to genes that have been found to be increased in frequency in SLE patients: sixth chromosomal markers such as histocompatibility leukocyte antigens (HLA) (e.g., DR2, DR3), and/or C2 and C4 null alleles (3, 9, 11–15), low levels of erythrocyte CR1 receptors (16), and IgG allotypes (17). Suppressor cell defects have also been demonstrated in relatives of SLE patients (7), as have the idiotypic markers of anti-DNA antibodies found in SLE patients (18).

Defining genetic allelism from a quantitative assessment of a particular protein (viz., C2, C3bR) can be difficult (19). For some proteins (e.g., T cell receptors) no protein polymorphism has been detected by serological methods. The use of Southern blotting and restriction fragment length polymorphism (RFLP) analysis facilitates the more accurate definition of genetic markers in both these situations. Furthermore, RFLP analysis will often define genetic markers in closer association with a particular disease than does protein allotypy. This has been accomplished in insulin-dependent diabetes mellitus (IDDM) (20) and other diseases (21–25). Such an approach has been applied to SLE where many studies have demonstrated an association with DR3 (3, 9, 11). However, Dunckley et al. (26) using DR and DQ probes did not demonstrate any RFLP association with SLE.

As T cell receptors are also involved in immune responses, it is reasonable that they could also play a role in SLE. In NZB X NZW mice a deletion of the beta chain of the T cell receptor has been recognized (27, 28). However, its role in the disease process is uncertain. RFLP analysis of patients with SLE has failed to demonstrate any association with T cell receptor beta chain markers (29, 30) or linkage in five families (31). The present study was instituted to reexamine the possible association between T cell receptor alpha chain markers and SLE. Previously only five multiplex families but no unrelated SLE patients were studied. Therefore, we studied nine additional multiplex SLE families and 31 unrelated SLE patients with T alpha, beta, and gamma probes to determine whether there was any association of T cell receptor genetic markers and SLE, and whether studying another ethnic group of SLE multiplex families might show concordance of markers with disease.

Using Pst I genomic digestion, and a T alpha gene cDNA probe, we found an association between a RFLP and SLE in unrelated patients as well as in multiplex SLE families.

Methods

Patients. 31 unrelated Caucasian "American" SLE patients (29 female, 2 male) were studied. Approximately one-half were of Southern European ancestry, and the rest (equally) of English/Irish, Eastern European, and Jewish ancestry. They all had four or more American Rheumatism Association criteria for SLE (32). The severity of the disease was estimated in respect to renal, central nervous system, skin,

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1. *Abbreviations used in this paper:* HLA, histocompatibility leukocyte antigens; IDDM, insulin-dependent diabetes mellitus; RFLP, restriction fragment length polymorphism; SLE, systemic lupus erythematosus.

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vascular, hematological, and serous membrane involvement. Antibodies to Sm, RNP, Ro, and La were detected by counter immunoelectrophoresis (33). Individuals had been previously HLA typed (9). 34 unrelated normal American Caucasian laboratory personnel (18 female, 16 male) served as controls. Their ancestry was similar to that of the "American" patients.

Families. Five multiplex SLE "American" families (10 patients, 20 relatives) described previously (31) and nine "Mexican" families (22 patients, 38 relatives; 6 families with 2 or more siblings with SLE; 1 family with 3 siblings and a mother with SLE; 1 family with 2 siblings and 1 niece with SLE; and 1 family with a mother and a daughter with SLE) were studied. 13 normal Mexican individuals served as controls. HLA and Gm typing permitted elimination of paternity exclusion in all families.

RFLP analysis. Genomic DNA was isolated from the peripheral blood white cells of patients, relatives, and normals (34). 7.5–10 μg of DNA were digested with Pst I according to the manufacturer's recommendations (New England Biolab, Beverly, MA). The DNA was electrophoresed on 0.85% agarose gel in TAE buffer, and then transferred to nitrocellulose membranes by the Southern blot method (35).

Gels of interest were blotted in duplicate ("twin blots") according to the technique of Smith et al. (36). Furthermore, these blots were reused after removal of the probe by two 15-min incubations in boiling 0.1× (SSPE), 0.1% (SDS) (37). These techniques permitted exact comparison of reactivity of the same band with different probes when necessary.

A 1.3-kb cDNA probe, PGA5 for the alpha chain of the T cell receptor was kindly provided by Dr. J. D. Capra. PGA5 was developed from a complete alpha chain mRNA of a T cell line HPB-MLT (38, 39). The cDNA probe includes sequences for the leader, V, J, C, and untranslated portion (3′ UT). Fragmentation of this probe into these different regions was performed using specific restriction enzymes (Fig. 1). After gel electrophoresis separation, each fragment was obtained by electroelution (40).

The random primer labeling technique was used to radiolabel the full cDNA probe and fragments with ^{32}P (41). The hybridization was performed overnight as described (42). The filters were then washed, with a final stringency of 1.0× (SCC), 0.1% SDS, buffer at 50° for PGA5 and at 42° for its fragments. Bands were visualized by autoradiography after 1–4 d exposure (XAR Kodak film) with or without intensifier screen at −80°C.

Statistical analysis. RFLP pattern frequency between the different groups were compared in 2 × 2 contingency tables using the chi square test, with Yates' correction; when one of the expected values was < 5 the two tailed Fishers exact test was employed.

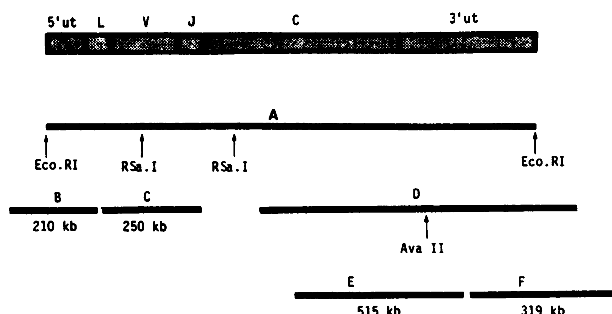

Figure 1. Representation of the PGA5 cDNA T cell receptor alpha gene. (*Top*) The 5′ untranslated (*5′ ut*), leader (*L*), variable (*V*), joining (*J*), constant (*C*), and 3′ untranslated (*3′ ut*) regions are indicated above the bar (61). (*Bottom*) The two steps of digestion of PGA5 allowing the location of the Pst I restriction sites are schematized: The complete probe (*A*) was first digested with Rsa I and Eco RI giving the indicated fragments *B, C,* and *D*. Subsequently the *D* fragment was cut with Ava II. Two fragments *E* and *F* were generated and hybridized onto the same blots as had *D* and the complete (*A*) probe.

Results

RFLP frequencies. 11 bands were recognized in different individuals with the complete cDNA probe; seven bands were polymorphic; there were 13 different combinations of bands (see Fig. 2 for representative patterns). Enzyme digestion of another DNA sample on individuals resulted in reproducibly identical RFLP patterns. Six of the 13 different combinations had the 1.3/3.0-kb band pair. All individuals had a 4.3-kb band. A combination of 1.3- and 3.0-kb bands was observed more frequently (15/31) in unrelated USA SLE patients than in normal controls (4/34) ($chi^2 = 8.81$; $P < 0.002$; relative risk, 7.03) (Table I). (If the P value, 0.002, is corrected by the number of polymorphic patterns not having the 1.3/3.0-kb pattern, i.e., 7, the corrected P value, 0.014, is still significant). Furthermore, these two bands were always associated in the same individual.

No significant associations were found between the 1.3/3.0-kb markers and any clinical, immunological pattern or HLA types (Table II), or with any European ethnicity.

Three of five USA families had the 1.3/3.0 band pattern. In one, bands were inherited from the father; concordance was observed between the two probands, and the one healthy sibling did not possess this pattern. In a second family the bands came from the mother and were found in the two siblings with SLE and two well siblings. The third family was not informative, as *all* individuals (both parents, two SLE siblings and three well siblings) had this band pattern. No individual homozygous for the 1.3/3.0 band pattern was recognized. The frequency of the 1.3/3.0 band pattern was not significantly different in patients (6/10) vs. relatives (9/20), but was significantly different vs. normal controls (4/34 ($chi^2 = 7.67$; $P < 0.0075$). The frequency in relatives was also significantly increased vs. normals ($chi^2 = 5.90$; $P < 0.02$).

Two of the nine Mexican families possessed this 1.3/3.0 band pattern, but were not informative for linkage; in one family all siblings (patients and well siblings) had the 1.3/3.0 band pattern and the father was not available; in the other family, while the two siblings and a niece had SLE the parents were not available for study. There was no significant difference in the frequency of the 1.3/3.0 band pattern in Mexican multiplex SLE patients (7/22) vs. relatives (8/38) vs. Mexican normals (0/13).

Location of the restriction sites for the 1.3/3.0 band pattern. To determine the location of the restriction site(s) determining the 1.3/3.0-kb band pattern, we carried out an RFLP analysis with cDNA fragments approximating the variable, joining, constant, and 3′ untranslated regions (see Fig. 1). The cDNA was cut with Rsa I and Eco RI and yielded an 834-bp fragment (constant and 3′ UT region) (*D*), a 210-bp fragment (5′ UT region) (*B*), 250-bp (V, J, C region) (*C*), and two smaller fragments (38). Genomic DNA from two American patients with the 1.3/3.0-kb band pattern, one control without, and one control with the pattern were tested with these fragments. The same "twin" blots were used for this purpose. The 250-bp fragment (*C*) of the VJC region hybridized with the 3.0-kb band and the 834-bp fragment (*D*) hybridized with the 1.3 and 3.0 bands (Fig. 3), suggesting that the restriction sites accounting for the 3.0- and 1.3-kb bands were in the vicinity of the constant and/or 3′ UT region. Therefore (see Fig. 1), the 834-bp fragment (*D*) was digested with Ava II to separate the "constant region" (515 bp) (*E*) from the "3′ untranslated" fragment (319 bp) (*F*) (38). Using these fragments as probes, the 515-bp

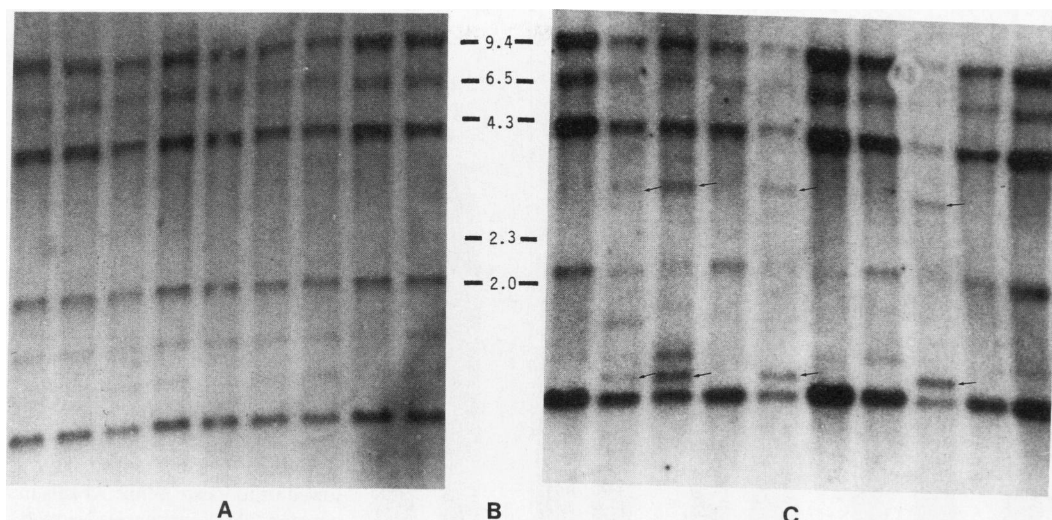

Figure 2. Representative polymorphic bands described in selected USA controls (*A*) and American SLE patients (*C*) after hybridization with the complete cDNA PGA5 probe. The 1.3/3.0-kb band pair (*arrows*) was found significantly more frequently in SLE patients. Hind III–digested fragments of lamba DNA were used as markers (*B*).

fragment (*E*) reacted with the 1.3-kb and the 3.0-kb band, while the 319-bp fragment (*F*) reacted with only the 1.3 band (Fig. 3).

Discussion

SLE is considered to be a genetically determined autoimmune disease; however, no genetic marker in strong association has been found. The association with HLA DR2 and DR3 only gave a relative risk of 3.27 and 3.2 (respectively) in a Caucasian population (43). However these markers were not always increased in frequency in all studies of SLE Caucasian patients (9, 44). Furthermore DR2 and DR3 are not increased in non-Caucasian SLE patients (45).

In the present study we have described a moderately strong (relative risk, 7) association between a RFLP pattern of the alpha chain gene of the T cell receptor and SLE in Caucasian American patients. This RFLP pattern, that is a 1.3-kb and 3.0-kb band pair, was recognized using a complete cDNA probe for the alpha chain, and Pst I digestion of genomic DNA. Furthermore, study of the USA families demonstrated concordance between SLE and this band pattern, in those three of five families who had this pattern, although some individuals without SLE also had the pattern.

In a previous study of these five multiplex SLE families, no concordance was observed of T alpha RFLP patterns and SLE (31). However, this lack of concordance was based on the lack of the concordance of all the RFLP bands. Based on the present study, the blots were reexamined, and indeed in three of the five families there was partial concordance between SLE and the 1.3/3.0 band pair. A study of nine Mexican families revealed that only two of nine families had the 1.3/3.0 band pattern. These two families were uninformative. Nevertheless possible interest was the observation that these two families had seven patients with SLE while the other seven Mexican families had only 15 SLE patients. This band pattern occurs both in Caucasian and Mexican SLE patients. Whether the frequency of its occurrence is the same in these two ethnic groups cannot be determined until more unrelated Mexican SLE patients and their appropriate controls and more Ameri-

Table I. Frequency 1.3/3.0-kb pair in American and Mexican SLE Patients and Controls

	1.3/3.0-kb pair	
	Present	Absent
Unrelated American patients	15	16
Multiplex American SLE patients	6	4
Multiplex American SLE relatives	9	11
American controls	4	30
Multiplex Mexican SLE patients	7	15
Multiplex Mexican SLE relatives	8	30
Mexican controls	0	13

Unrelated American patients vs. American controls: chi square = 8.81; relative risk = 7.03; $P < 0.002$. Multiplex American patients vs. American controls: chi square = 7.67; relative risk = 11.25; $P < 0.0075$.

Table II. Association of RFLP with Genetic and Immune Markers in American SLE Patients

	1.3/3.0-kb band	
	Present	Absent
DR2 present	5	3
DR2 absent	23	26
DR3 present	5	10
DR3 absent	10	6
Anti-Sm present	5	2
Anti-Sm absent	10	14
Anti-RNP present	5	4
Anti-RNP absent	10	10
Anti-Ro present	3	4
Anti-Ro absent	12	12
Anti-La present	3	3
Anti-La absent	12	13
Anti-DNA present	13	12
Anti-DNA absent	2	4

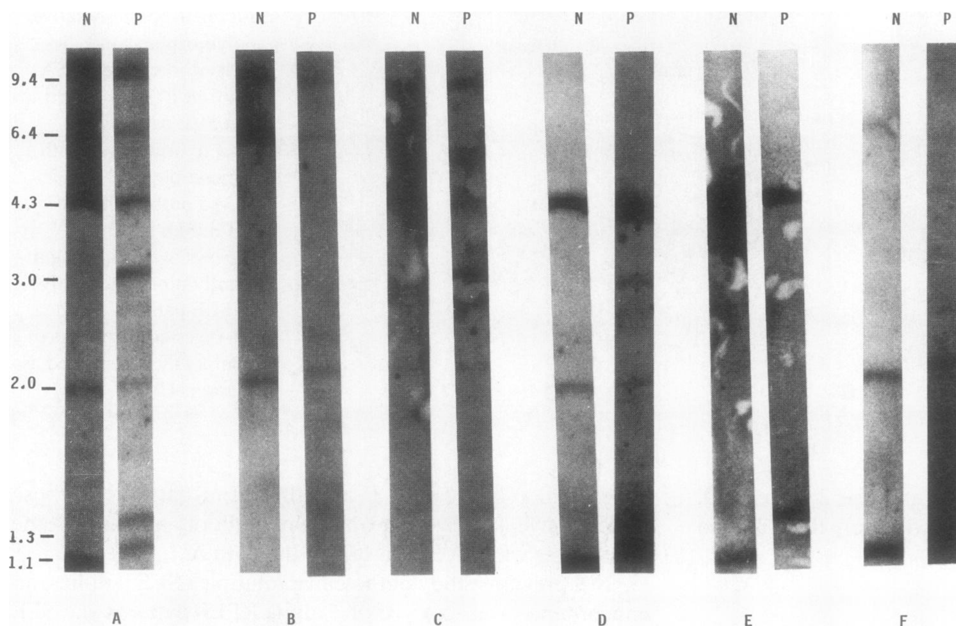

Figure 3. Location of the Pst I restriction site in the constant region giving the 1.3/3.0-kb band pair. The PGA-5 cDNA probe and its five fragments, obtained as indicated in Fig. 1, were hybridized successively onto the same genomic DNAs. DNA from a normal control without the 1.3/3.0 (*N*) and DNA from a SLE patient (*P*) with the bands of interest were digested, electrophoresed, and blotted along with a control of molecular weight (data not shown) according to the "twin blot" technic. The blots were then hybridized with fragments *A–F* (derived as per Fig. 1). The different steps allowed us to locate all the RFLPs in a region. No discrepancies were observed between *P* and *N* for the common bands. The 3.0-kb band was only seen with the *C* and *D* fragments, whereas the 1.3-kb band was noted with the *D, E,* and *F* fragments. There is an artifact at the 3.0-kb location in the blot probed with the *E* fragment.

can multiplex families and unrelated American patients can be studied.

Analysis of nine Mexican families by RFLP using Bgl II and Pst I digestion of genomic DNA and beta and gamma chain probes of the T cell receptor demonstrated no concordance of SLE with any RFLP pattern (data not shown).

The location of the restriction sites resulting in the 1.3/3.0-kb band patterns appears to be in the constant region. T cell receptor constant region genes have also been associated with multiple sclerosis and myasthenia gravis sites (46). No apparent association between this TCR alpha chain RFLP and HLA-DR alleles nor with specific antinuclear antibodies was observed. However, Frank et al. have recently demonstrated an association of a pair of RFLPs in the CB1 and CB2 regions of the TCR beta chain with anti-Ro. In addition anti-Ro was associated with DQ1/DQ2 heterozygosity, suggesting possible interaction of TCR and MHC loci in promoting anti-Ro antibody formation (47). The role of this region of the alpha chain of the T chain receptor in disease determination needs to be established.

No apparent association between the presence of the 1.3/3.0 band pattern and clinical and immunological features of SLE and HLA types were noted. Weak associations have been observed between IDDM, Graves disease, hypothyroidism, multiple sclerosis, myasthenia gravis, membranous nephropathy, and rheumatoid arthritis and RFLPs associated with the alpha and beta chains of the T cell receptor (48–59). Our results differ from that of two other studies that examined a possible association between SLE and T cell receptor genes. Dunckley et al. did not observe any association of SLE with RFLP patterns using Eco RV digestion of genomic DNA and a T alpha probe (and other enzymes and T beta and T gamma probes) (60). In a previous study from this laboratory of five multiplex SLE families and a Bgl II and Pst I RFLP analysis using the T alpha probe no concordance was observed between SLE probands in the same family and any RFLP pattern. Therefore, we had concluded that there was no (recessive) SLE susceptibility gene in the vicinity of the alpha chain of the T cell receptor (31). However, in that study we had focused on the entire polymorphic RFLP pattern; in the present study after we had recognized the 1.3/3.0 band pattern in the nonmultiplex family SLE patients, we reexamined these five SLE families and found the 1.3/3.0-kb band pattern partially concordant with disease transmission in three (families). These observations, therefore, do not exclude dominant transmission. More multiplex families need to be studied to resolve this question.

The 1.3/3.0-kb pattern appeared seldom in our normal Caucasian American population. Using the Hardy-Weinberg equation the 1.3/3.0 kb has an estimated gene frequency of 0.06. The infrequency of the heterozygous 1.3/3.0 band pattern state no doubt explains our lack of finding any homozygous individuals (expected frequency 0.004). The gene frequency of the 1.3/3.0-kb pattern in the American Caucasian SLE patients was 0.49; therefore two homozygotes might have been detected. However, none were observed. Based on the map of T alpha (38, 39, 61, 62, 63), and the present observations, we propose that the allele for the 1.3/3.0-kb pattern is a 4.3-kb band (Fig. 4). Because we never observed a homozygous 1.3/3.0-kb pattern this hypothesis must remain just that. However, the observation of 4.3-, 3.0-, and 1.3-kb RFLP bands fits the mapping. In addition, one cannot completely exclude the superposition of (another) allelic 4.3-kb band. This possibility could then explain the lack of appearance of the homozygous pattern, as the band would be hidden. More families informative for the 1.3/3.0-kb pattern will have to be studied to confirm this hypothesis. In contrast, the frequency of the different RFLP patterns with Eco RV (1.7 kb = 0.24/1.6 kb = 0.76) and with Bgl II (3.2 kb = 0.90/2.9 kb = 0.1) are greater. The low frequency of the 1.3/3.0 suggests a relatively

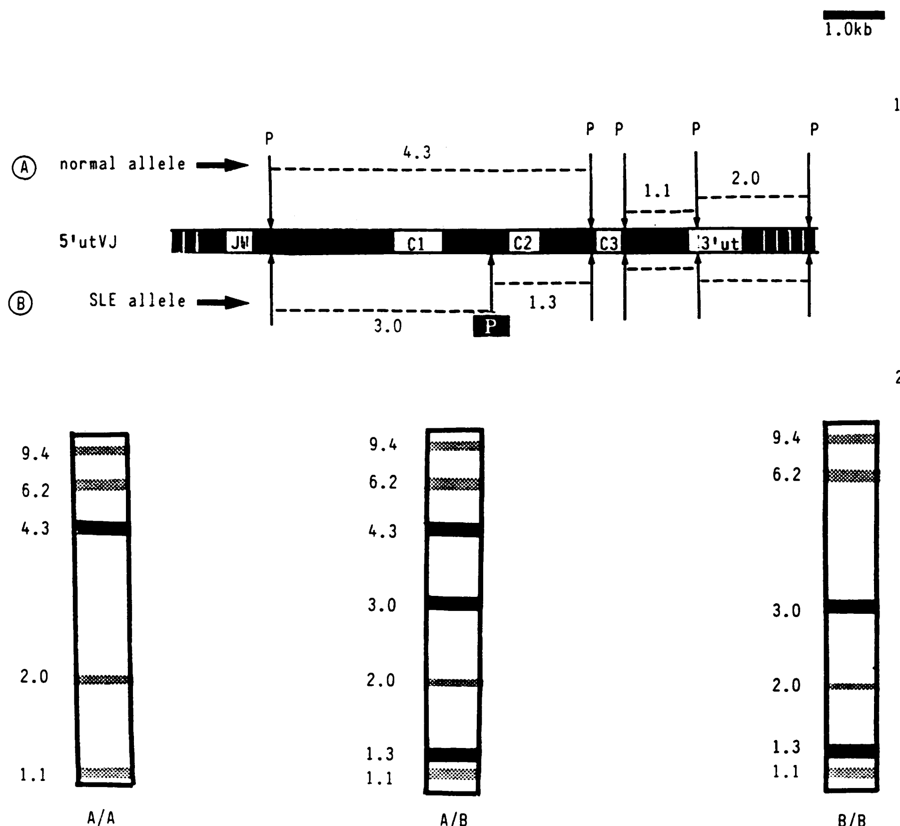

Figure 4. Proposed mapping of the Pst I restriction sites based on the RFLP data of Fig. 3 and Yoshikai et al. (61). Only the simplified constant region showing the different exons of interest (wide boxes) and principal Pst I restriction sites (p_1 vertical arrows) are shown (61). Sizes of the bands of interest are indicated above and below the dashed lines. The restriction site (bold *P*) is located between the C1 and C2 region. The different RFLP pattern of the proposed homozygous (*A/A*), heterozygous (*A/B*), and homozygous (*B/B*) are then represented below. Dotted bands are the common bands and full bands are the polymorphic bands of interest.

recent mutation and/or a disadvantage for this RFLP pattern in terms of evolution (61). The morbidity of the SLE could be this disadvantage. The difference between our work and that of others could also be explained by the possible complexity of different genetic markers in SLE (43). In some SLE patients, the disease may be more associated with DR2, others DR3, others T alpha genes. The presumed T alpha chain SLE susceptibility gene may have different forms (and/or alleles) which have different degrees of penetrance, in causing SLE, based on other factors in their environment.

In conclusion, a Pst I RFLP using a T alpha chain receptor probe was found strongly associated with SLE. The role of this marker in the disease process and a more precise definition of its location will require further studies.

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