lack of contact inhibition. See (*22*) for methods of oncogene incorporation and clonal selection.
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18. Swiss 3T3 fibroblasts were obtained from H. Green (Department of Physiology and Biophysics, Harvard Medical School) and cultured in Dulbecco's modified Eagle's medium and 10% fetal calf serum. For the whole-cell recordings, individual cells were obtained by brief (5 to 10 minutes) trypsinization (0.05% trypsin + 0.5 m*M* EDTA). The cell suspension was allowed to settle in the recording chamber, and currents were measured from individual cells not connected to their neighbors.
19. Cell-attached recordings were obtained from cells in cultures without trypsinization as well as from the dissociated cells used for whole-cell recordings. We noticed no difference in the unitary channel properties between the two groups.
20. The conductance of the L-type Ca^{2+} channel with 110 m*M* $BaCl_2$ was 25 pS. The conductance of the T-type Ca^{2+} channel with 110 m*M* $CaCl_2$ was 8 pS. Unlike the L-type channel (*6, 21*), the T-type channel had similar conductance and kinetics with either Ca^{2+} or Ba^{2+} as the charge carrier (*6, 18*).
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22. The cell lines were grown from G418-resistant colonies selected after infection with a recombinant retrovirus (*23*) carrying the neomycin/G418 resistance gene and the following inserts: (i) no second gene (control virus-infected cells); (ii) activated c-H-*ras*-1 complementary DNA–encoding leucine in place of glutamine at codon 61 [M. J. Corbley, unpublished data; (*24*)]; (iii) v-*fms*, the transforming gene of the McDonough strain of feline sarcoma virus (*25*); and (iv) polyoma virus middle tumor antigen (middle T) (*26*). NIH 3T3 cells were also grown from a transformed focus after transfection with EJ-*ras* DNA (*27*). The results from cells infected with activated c-H-*ras* ($n = 10$) or transfected by EJ-*ras* ($n = 7$) were similar and were therefore pooled and are shown as "RAS" in Fig. 3.
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28. We found no difference in the whole-cell Ca^{2+} currents of virus-infected NIH 3T3, noninfected NIH 3T3, and noninfected Swiss 3T3 cells.
29. We thank D. K. Morrison and V. Cherington for providing cell lines and H. Green and O. Kehinde for cells, advice, and use of their culture facilities. Supported by grants from the U.S. PHS (HL37124 to P.H.; CA21082 to T.M.R.) and the American Cancer Society (CD342 to P.H.).

22 October 1987; accepted 7 January 1988

A Newly Characterized HLA DQ_β Allele Associated with Pemphigus Vulgaris

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The inheritance of particular alleles of major histocompatibility complex class II genes increases the risk for various human autoimmune diseases; however, only a small percentage of individuals having an allele associated with susceptibility develop disease. The identification of allelic variants more precisely correlated with disease susceptibility would greatly facilitate clinical screening and diagnosis. Oligonucleotide-primed gene amplification in vitro was used to determine the nucleotide sequence of a class II variant found almost exclusively in patients with the autoimmune skin disease pemphigus vulgaris. In addition to clinical implications, the disease-restricted distribution of this variant should provide insight into the molecular mechanisms underlying associations between diseases and HLA–class II genes.

THERE IS A STRIKING GENETIC ASSOCIATION between susceptibility to human autoimmune disease and particular alleles of the major histocompatibility complex (HLA) class II (D) region (*1*). This multigene complex on chromosome 6 encodes the α and β polypeptides of the highly polymorphic DP, DQ, and DR heterodimers that regulate immune responsiveness (*2*). Given the significant effects of class II protein sequence variation on the specificity of the immune response in mice (*3*), there have been intensive efforts to identify autoimmune disease–unique or restricted class II allelic variants by serologic, cellular, and restriction fragment length polymorphism (RFLP) typing methods (*4*). Two DQ_β RFLP variants that very clearly distinguish between patients with the autoimmune skin disease pemphigus vulgaris (PV) and class II–matched healthy controls were recently identified (*5*). We now report the first domain nucleotide sequence of both of these RFLP variants. One of these sequences represents a previously unidentified DQ_β allele present in 13 of 13 DRw6,DQw1 Israeli PV patients but in only 1 of 13 DR- and DQ-matched Israeli controls.

Pemphigus vulgaris, a severe autoimmune disease of the skin characterized by intraepidermal blistering, is mediated by autoantibodies to an epidermal cell-surface protein (*6*). The disease is relatively frequent among Jews (*7*); it is strongly associated with the DR4 serologic specificity among Ashkenazi Jews and with the DR4 and DRw6 serologic specificities in non-Ashkenazi Jews (*5*). However, the association of PV, by RFLP analysis, with DQ_β rather than DQ_α or DR_β (*5*) indicates that DQ_β may be the susceptibility locus. The previously reported DR associations might then reflect the known strong linkage disequilibrium between the DR and DQ loci. There are four serologically identified DQ alleles: DQw1, DQw2, DQw3, and DQWa (or DQwBLANK) (*8*). DR4 haplotypes are nearly always associated with DQw3, whereas most DRw6 haplotypes have the DQw1 specificity (*9*). The DQw1 serologic determinant is also found in DR1 and DR2 haplotypes, but DR1-, DR2-, and DRw6-associated DQw1 molecules have been differentiated at the protein and RFLP levels (*10*). Furthermore, there is DQ heterogeneity within the DRw6 haplotype (*11*) (Table 1).

When Bam HI–digested genomic DNA from several $DQw1^+$ PV patients and controls was examined by Southern blot and hybridized with a DQ_β complementary DNA (cDNA) probe, the PV-associated 2.5-kb fragment (*5*) was found in three of three DRw6,DQw1 PV patients (Fig. 1, lanes 4, 6, and 10), three of three DR1,DQw1 homozygous typing cells (HTC) (lanes 1 to 3), one of three DR2,DQw1 HTC (lanes 11 to 13), and none of three DRw6,DQw1 HTC (lanes 7 to 9). The presence of this fragment in the DR1,DQw1 HTC and in the AZH HTC, an example of the non-Dw2, non-Dw12 DR2 subtype (lane 11), likely reflects the near identity of these DQ_β alleles at the

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nucleotide level (*12*). This raised the possibility that the apparent disease specificity of the Bam HI 2.5-kb RFLP represented an increase in the frequency of a previously sequenced DQw1$_\beta$ allele in DRw6,DQw1 PV patients relative to DRw6,DQw1–matched controls. Therefore, it was necessary to determine the nucleotide sequence of the DQ$_\beta$ allele from a DRw6,DQw1 PV patient.

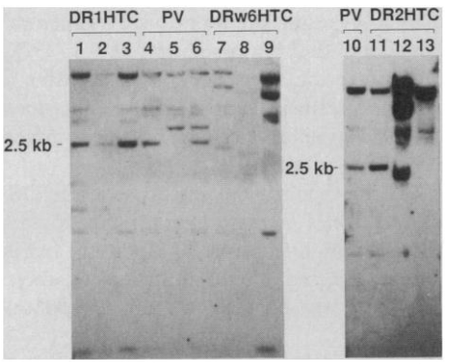

Fig. 1. DQ$_\beta$ RFLP pattern of Bam HI–digested DNA from PV patients and DQw1$^+$ HTC. The disease-associated 2.5-kb band is indicated. (Lanes 1 to 3) DR1,DQw1 HTC: MVL, BVR, and IBW4; (lanes 4 to 6) PV patients: DR4,DQw3/DRw6,DQw1, DR4,DQw3/DR5,DQw3, and DR5,DQw3/DRw6,DQw1; (lanes 7 to 9) DRw6,DQw1 HTC: WVD, APD, and WT46; (lane 10) PV patient: DR4,DQw3/DRw6, DQw1; and (lanes 11 to 13) DR2,DQw1 HTC: AZH (non-Dw2, non-Dw12), PGF (Dw2), and BGE (Dw12). Genomic DNA was extracted from lymphoblastoid cell lines, digested (10 μg) with Bam HI, transferred to nylon membrane (Amersham) by Southern technique, and probed with a DQ$_\beta$ fragment (^{32}P-labeled by hexamer priming) as described (*26*).

Because most of the variability among class II alleles is confined to the first extracellular domain (*13*), we used a rapid in vitro DNA amplification technique (*14*) to produce large quantities of a 360–base pair first-domain fragment, which was then gel-purified and directly cloned into the M13 sequencing vector. The substrate for amplification was cDNA synthesized from total RNA (*15*) of a DR4,DQw3/DRw6,DQw1 PV lymphoblastoid cell line. This patient had both the DRw6 and DR4 DQ$_\beta$ RFLPs associated with PV. Two different DQ$_\beta$ nucleotide sequences were obtained. One was identical to a previously published DQw3.2$_\beta$ sequence (*16*) (Fig. 2) and represented the DR4-associated DQ$_\beta$ allele. The second was a new DQ$_\beta$ allele identical in predicted amino acid sequence to DR1,DQw1.1 and DR2,DQw1.AZH except at codon 57, where Asp (GAC), rather than Val (GTT) or Ser (AGC), was present (Fig. 2). Comparison with other published DQ$_\beta$ nucleotide sequences revealed that the DR2,DQw1.12 sequence is identical to the newly identified DQ$_\beta$ sequence for a 44–base pair stretch that includes codon 57 (Fig. 2) and thus may have served as a donor sequence to generate the new DQ$_\beta$ allele from an ancestral DQw1.1$_\beta$ allele by a gene conversion–like event. Much evidence has accumulated for such a mechanism in the generation of class II allelic polymorphism, and it has been proposed that such "epitope shuffling" could generate allelic variants that predispose to certain autoimmune diseases (*3, 17, 18*). Subsequent to identifying the DQ$_\beta$ variant associated with PV, we found that the DRw6,DQw1 non-Israeli HTC WT52 expresses an identical first domain DQ$_\beta$ amino acid sequence (*15*). By cellular typing WT52 is Dw9, and we have therefore designated the PV-associated DQ$_\beta$ allele as DRw6,DQw1.9.

To determine the frequency of the new DQ$_\beta$ allele in PV patients and matched Israeli healthy controls we designed the PV6$_\beta$ allele-specific oligonucleotide (ASO) probe [5′-GGCGGCCTGACGCCGAG-3′; nucleotides 161 to 177 (boxed) in Fig. 2], which differs sufficiently from all other DQ$_\beta$ alleles (except DR2,DQw1.12; Fig. 2) that it can be washed off under high-stringency conditions. This probe was used in a dot blot hybridization assay with in vitro–amplified genomic DNA of several DRw6,DQw1–positive and DRw6,DQw1–negative PV patients and controls. A representative example is shown in Fig. 3 (top). The PV6$_\beta$ ASO probe specifically hybridized to amplified DNA from DRw6, DQw1 PV patients, but not to DRw6, DQw1, or DR1,DQw1 HTC, or to non–DRw6,DQw1 PV patients. The presence of a variant DQ$_\beta$ sequence in DQw1 PV patients was confirmed by lack of hybridization (Fig. 3, bottom) with the DQw1.1$_\beta$(54)

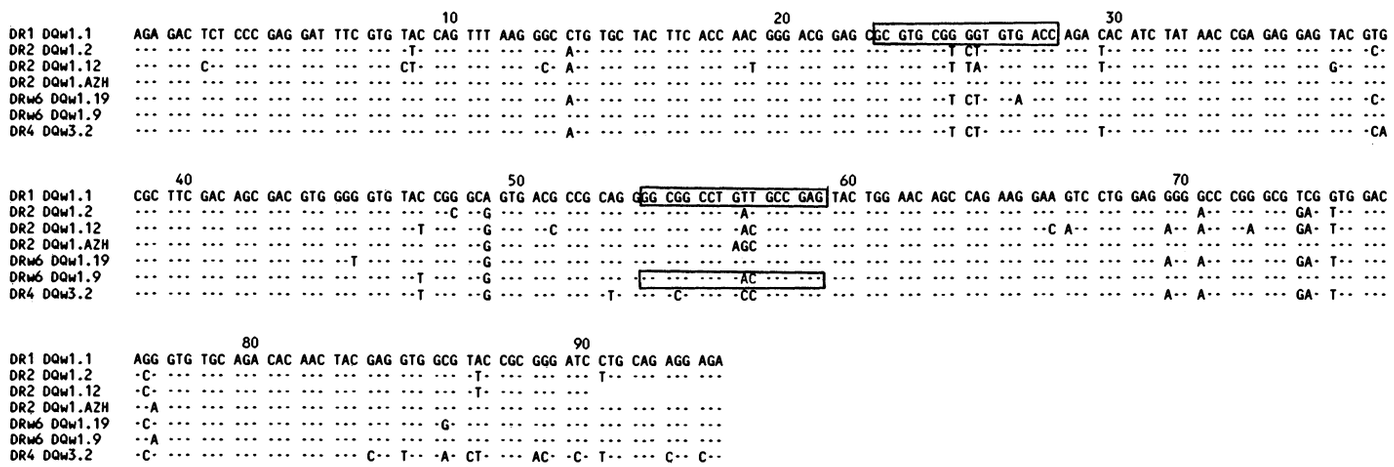

Fig. 2. DQ$_\beta$ first-domain nucleotide sequence of a DRw6,DQw1 PV patient (DRw6,DQw1.9) compared to normal DQw1 haplotypes. The normal DQw1 sequences are from the following cell lines: DR1,DQw1.1 LGL (*23*); DR2,DQw1.2, PGF (*12*); DR2,DQw1.12, BGE (*12*); DR2, DQw1.AZH, AZH (*12*); DRw6,DQw1.19, Daudi (*21*). The DQ$_\beta$ first-domain nucleotide sequence of a DR4,DQw3 PV patient is shown on the last line (DR4,DQw3.2) and is identical to the DQ$_\beta$3.2 subtype of DR4 (*16*). DR2,DQw1.2 and DRw6,DQw1.18 are identical at the amino acid level except at codon 30 (Tyr and His, respectively) (*15*). The Daudi cell line is homozygous for the DRw6 and DQw1 serological specificities, but has both DQw1.18 and DQw1.19 DQ$_\beta$ alleles (*21*). Complementary DNA was synthesized (*27*) from total cellular RNA of a lymphoblastoid cell line established from a DR4,DQw3/DRw6,DQw1 PV patient and amplified in vitro as described (*14, 15*) with DNA polymerase I Klenow fragment (BRL or Pharmacia) in two rounds of 15 cycles separated by purification by phenol-chloroform extractions. The DQ$_\beta$ oligonucleotide primers were LPDQβ3, 5′-GAGCACCCCAGTGGCTGAGGG-3′ [codons −8 to −1 according to the numbering system of Larhammar *et al.* (*16*)] and AMPDQβ7, 5′-GTTGTGGTGGTTGAGGGCCTC-3′ (codons 107 to 113). Extension and annealing reactions were done at 37°C. The amplified product was gel-purified using NA-45 paper (Schliecher and Schnell) as described (*15*). One-tenth of the amplified DNA was directly blunt-end ligated with T4 ligase (Biolabs) into the M13 sequencing vector [identical to M13K8.2 (*28*) except that an Eco RV site replaces the Sma I site]. Transformation into *Escherichia coli* JM101 gave 100 to 200 M13 plaques, which were directly sequenced. Seventy to 90 percent of the plaques contained class II sequences. More than one clone for each sequence was isolated and sequenced on both strands.

ASO probe [5′-GGCGGCCTGTTGCC-GAG-3′; nucleotides 161 to 177 (boxed) in Fig. 2]. The failure of this ASO probe to hybridize with DNA from the DRw6,DQw1 lines WVD and APD was expected since they do not have the DQw1.1$_\beta$ RFLP pattern (Fig. 1) (*11*). Finally, the presence of a DQw1.1-related rather than a DR2, DQw1.12 DQ$_\beta$ allele in DRw6,DQw1 PV patients was confirmed by hybridization with the DQw1.1$_\beta$(23) ASO probe [5′-GCG-TGCGGGGTGTGACC-3′; nucleotides 68 to 84 (boxed) in Fig. 2].

Results of a more extensive survey of PV patients and controls for the presence of the DRw6,DQw1.9 DQ$_\beta$ allele are summarized in Table 2. Dot blot hybridization of amplified genomic DNA with the PV6$_\beta$, DQw1.1$_\beta$(54), and DQw1.1$_\beta$(23) ASO probes demonstrated that 13 of 13 DRw6,DQw1 Israeli PV patients (including Ashkenazi and non-Ashkenazi Jews) and 1 of 13 DRw6,DQw1 healthy Israeli controls had the DRw6,DQw1.9 DQ$_\beta$ allele. Furthermore, none of 14 DRw6,DQw1–negative PV patients, none of 5 DR4, DQw3/DR1,DQw1 PV patients, and none of 5 DR4,DQw3/DR1,DQw1 healthy controls had this DQw1$_\beta$ variant. These results indicate that the PV-associated DQ$_\beta$ allele is significantly overrepresented in DRw6, DQw1 PV patients relative to matched healthy controls.

The striking association of the new DQ$_\beta$ allele with PV has implications for clinical screening and diagnosis. Dot blot hybridization with the PV6$_\beta$ ASO probe may prove useful for distinguishing PV from other pemphigus-like diseases. Furthermore, since the onset of PV occurs well into adulthood, early screening for the presence of the PV-associated DQ$_\beta$ variant may provide an opportunity for prophylactic intervention. Screening for the PV-associated Bam HI 2.5-kb RFLP would not be informative since DR1 and DR2(AZH) haplotypes (not associated with PV) also have this RFLP but differ from DRw6,DQw1.9 at codon 57. The identity of residue 57 was recently shown to be strongly correlated with susceptibility to insulin-dependent diabetes mellitus in humans and mice (*15*). Correlation of disease susceptibility with residue 57 is now further extended and may reflect its importance in the function of the DQ molecule. Clearly, however, the entire DQ$_\beta$ molecule is relevant for disease susceptibility; DR2,DQw1.2 and DR2,DQw1.12, which are identical to DRw6,DQw1.9 at amino acids 39 to 70 and 38 to 65, respectively, but show multiple differences throughout other areas of the first domain, are not associated with PV. It is also possible that other closely linked loci (class II or non-HLA) may contribute to or be responsible for disease susceptibility on a haplotype marked by the new PV-associated DQ$_\beta$ allele. Our results do not provide a structural basis for the PV-associated DR4 DQ$_\beta$ RFLP variant. This RFLP may reflect a polymorphism in HLA-DP or in the noncoding regions that regulate the cell-surface expression of class II.

The mechanism by which particular DR or DQ alleles increase susceptibility to certain autoimmune diseases is unknown, but is perhaps related to the function of these molecules in normal immune responses

Table 1. Subtypes of DR2, DR4, and DRw6 haplotypes. The DR2, DR4, and DRw6 haplotypes have been subdivided by cellular typing methods. The subtypes are referred to as Dw alleles but in most cases it is not known which locus or loci (DR, DQ, or DP) encode the Dw specificities. Not all of the DQw1 subtypes indicated can be distinguished serologically. The DRw6,DQw1.9 and DR1,DQw1.1 DQ$_\beta$ alleles are identical by RFLP analysis (*11*). By RFLP analysis, the DR2,DQw1.2 and DRw6, DQw1.18 DQ$_\beta$ alleles are identical (*11*). The DQw1.19 DQ$_\beta$ allele is also found on some DR2 haplotypes (*11, 21*). The Raji HTC has been typed serologically as DRw6/DR3 and is identical at the amino acid level to DR1,DQw1.1 at DQ$_\beta$ (*22, 23*). However, recently it has been shown that Raji has a DQ$_\beta$ RFLP pattern found in DRw10 individuals (*24*).

DR	Dw	DQ
2	2	w1.2
2	12	w1.12
2	AZH	w1.AZH
4	4	w3.1*
4	4, 10, 14	w3.2
4	15	wBLANK
w6	9	w1.9
w6	18	w1.18
w6	19	w1.19

*Three DQ$_\beta$ alleles have been sequenced from DR4-positive haplotypes: DQw3.1, DQw3.2, and DQwBLANK (*15, 25*).

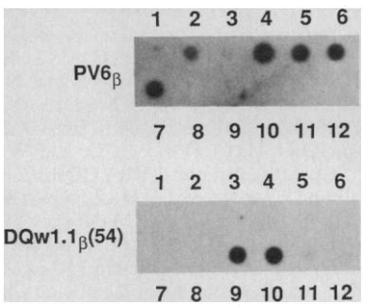

Fig. 3. Dot blot hybridization of amplified genomic DNA from PV patients and controls with PV6$_\beta$ and DQw1.1$_\beta$(54) ASO probes. (Samples 1 to 8) PV patients: DR4,DQw3/DR5,DQw3, DR5,DQw3/DRw6,DQw1, DR4,DQw3/DR5, DQw3, DR5,DQw3/DRw6,DQw1, DR4, DQw3/DRw6,DQw1, DR5,DQw3/DRw6, DQw1, DR5,DQw3/DRw6,DQw1, and DR5, DQw3/DR7,DQw2; (samples 9 and 10) DR1,DQw1 HTC: MVL and BVR; and (samples 11 and 12) DRw6,DQw1 HTC: WVD and APD. Genomic DNA was extracted from lymphoblastoid cell lines and amplified for 28 continuous cycles by using the reaction conditions described in Fig. 2 and the oligonucleotide primers GLPDQβ1, 5′-GATTTCGTGTACCAGTTTAAGGGC-3′ (amino acid positions 6 to 13) and GAMPDQXβ2, 5′CCACCTCGTAGTTGTGTCTGCA-3′ (amino acid positions 79 to 86). One-fourth of the amplified product was applied to nitrocellulose paper with a BRL Hybri-Dot manifold, and the paper was then baked for 2 to 3 hours at 80°C. Filters were prehybridized in 6× SSC and 0.5 percent SDS containing yeast transfer RNA (250 μg/ml) and 5× Denhardt's solution for at least 10 minutes at 42°C, then hybridized 12 to 16 hours with 3×10^6 to 6×10^6 cpm of ^{32}P-labeled ASO at 42°C (*15*). After hybridization, blots were washed in 6× SSC and 0.1 percent SDS at room temperature for 10 minutes and placed under x-ray film for 4 hours to ensure that each well contained amplified DNA. Blots were then washed in 6× SSC and 0.5 percent SDS at a temperature (T_d), equal to (the number of GC base pairs × 4) + (the number of AT base pairs × 2), so that the ASO probe would be removed from target sequences with one or more base pair mismatches. The washing temperatures for each oligonucleotide were: PV6$_\beta$, 61°C; DQ$_\beta$1.1(54), 61°C; and DQ$_\beta$1.1(23), 59°C.

Table 2. Frequency of the DRw6,DQw1.9 DQ$_\beta$ allele in PV patients and controls. Dot blot hybridization was carried out with the PV6$_\beta$ and DQw1.1$_\beta$(54) ASO probes as described in the legend to Fig. 3. All samples that hybridized with the PV6$_\beta$ ASO probe also hybridized with the DQw1.1$_\beta$(23) ASO probe, confirming the presence of a DQw1.1–related, rather than a DR2, DQw1.12–related allele. The DRw6,DQw1 group included six DR4,DQw3/DRw6,DQw1 patients, six DR5,DQw3/DRw6,DQw1 patients, and one DRw6,DQw1/− patient. All of these patients carried the Bam HI 2.5-kb RFLP. Of the 13 DRw6,DQw1 healthy controls, the DR4,DQw3/DRw6,DQw1 healthy control that had the DRw6,DQw1.9 DQ$_\beta$ sequence was the only one to carry the Bam HI 2.5-kb RFLP. This individual, who is presently young and disease-free, will be interesting to follow clinically in later years. The DR4,DQw3 group included only DQw1-negative patients. Six were DR4, DQw3/−, seven were DR4,DQw3/DR5,DQw3, and one was DR4,DQw3/DR9,DQw3. None of the patients or controls in this group carried the Bam HI 2.5-kb RFLP. All of the patients and controls in the DR4,DQw3/DR1,DQw1 group carried the Bam HI 2.5-kb RFLP.

Group	PV patients		Controls	
	PV6$_\beta$	DQw1.1$_\beta$(54)	PV6$_\beta$	DQw1.1$_\beta$(54)
DRw6,DQw1	13/13	0/13	1/13	3/13
DR4,DQw3	0/14	0/14	0/9	0/9
DR4,DQw3/DR1,DQw1	0/5	5/5	0/5	5/5

(*15*). Intrathymically, stromal cell–associated class II molecules interact with T lymphocyte precursors to establish self tolerance and determine the specificity of the mature T cell repertoire (*19*). In peripheral lymphoid organs, class II molecules bind antigen fragments to form a bimolecular complex, which can then be recognized by the antigen receptor on $CD4^+CD8^-$ T cells (*20*). A variant class II molecule could thus contribute to autoimmune disease susceptibility by influencing T cell repertoire development or by altering antigen-binding properties. The latter possibility could be tested if sufficient quantities of the putative epidermal cell PV autoantigen become available.

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29. A.A.S. is a Centennial fellow of the Medical Research Council of Canada and is a recipient of an independent research allowance from the Alberta Heritage Foundation for Medical Research. J.A.T. gratefully acknowledges fellowships from the European Molecular Biology Organization and the Science and Engineering Research Council. This work was supported by the National Institutes of Health. We thank P. Travers, C. Jacob, and Z. Fronek for helpful discussions and critical review of the manuscript, B. Lee and P. Patek for reagents, M. Trucco for data prior to publication, and K. Karagianes and K. Moody for final preparation of the manuscript.

16 September 1987; accepted 19 January 1988

Atrial Natriuretic Factor–SV40 T Antigen Transgenes Produce Tumors and Cardiac Arrhythmias in Mice

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Transgenic mice that carry fusions between the transcriptional regulatory sequences of atrial natriuretic factor (a hormone intimately involved in the regulation of blood pressure) and those encoding SV40 T antigen (an oncoprotein) were generated. Although both atria express the fusion gene, the pathological response to T antigen is asymmetrical. The right atrium undergoes a several hundredfold increase in mass while the left atrium remains relatively normal in size. Hyperplasia is accompanied by a progressive increase in both the frequency and severity of abnormalities in the atrial conduction system, which ultimately result in death.

NORMAL HEART FUNCTION IS DEpendent on the coordinated depolarization and contraction of atrial and ventricle myocardiocytes. Abnormalities in this conduction system can impair cardiac function, often with catastrophic effects. Although it is possible to generate conduction abnormalities in animals by pharmacological or surgical procedures, there are no model systems that exhibit heritable pathology.

Atrial natriuretic factor (ANF), a peptide hormone that is synthesized and stored in the cardiac atria, is a major participant in the systemic regulation of extracellular fluid volume and electrolytes. Secretion of ANF into the blood stream elicits physiological responses, such as natriuresis and a concomitant decrease in blood pressure (*1*). The high abundance of ANF in the atria has facilitated the molecular cloning of ANF complementary DNA's, and subsequently genomic clones (*2*). The ANF gene is highly conserved between species and is divided between three exons and two introns.

The technique of introducing genes into the germ line of mice permits the simultaneous functional analysis of promoter sequences in every cell type of a given animal (*3–5*). The sequences required for the transcriptional regulation of the ANF gene in vivo were identified as follows: transgenic mice that carry fusions between the putative ANF promoter and the SV40 large T antigen oncogene (ANF-TAG) were generated. The fusion gene carried the segment at −500 to +77 bp relative to the ANF messenger RNA cap site, and a 2.7-kbp restriction fragment encompassing the SV40 early region. Orientation was such that transcripts originating from the ANF promoter would encode the oncoprotein. The fusion gene was microinjected into embryos, and transgenic mice were generated and screened (*6*). Eight ANF-TAG transgenic mice (Table 1) were subdivided into two groups on the basis of their relative longevity. The ANF-TAG's 24, 25, 52, 84, and 210 each survived long enough to generate progeny litters of mice, and in four out of five instances have transmitted their respective transgenes. In each case, the founder mouse survived at least 22 weeks before it died as a consequence of transgene expression. Conversely, ANF-TAG's 49, 54, and 75 died shortly after birth and hence no lineages could be established.

Atrial expression of the ANF-TAG fusion gene was demonstrated by Western blot analyses (Fig. 1A). An approximately 90-kD polypeptide that comigrates with COS-1–derived SV40 T antigen is present in the atria from an ANF-TAG 210 mouse, but not in nontransgenic atria. Similar results were obtained with the other ANF-TAG transgenic lines. Western blot analyses were performed to ascertain the fidelity of the

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