body of mammalian eyes also contain high concentrations of HA and are avascular throughout most of life. The association of large concentrations of HA with these avascular tissues may indicate a continuing role for HA in establishing and maintaining avascularity.

Extracellular matrix molecules, particularly hyaluronate, are believed to participate in various developmental processes (*15*). We have correlated the presence of increased levels of hyaluronate with the absence of blood vessels. If HA acts in other regions of the embryo as it appears to act in the peripheral mesoderm of the chicken wing bud, it will influence the location of embryonic capillary networks. Since all of the major blood vessels are formed from such capillary networks, HA may have a critical role in determining the location of these vessels during normal development.

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Somatic Mutations of Immunoglobulin Variable Genes Are Restricted to the Rearranged V Gene

Abstract. *An important question concerning the mechanism of somatic mutation of immunoglobulin variable (V) genes is whether it involves all of the numerous V genes in a differentiated B cell, independent of location, or if it is restricted to a particular chromosomal site. Comparison of the sequence of two alleles of a given V gene shows that the mutations are limited to the rearranged V gene, while the same V gene on the other chromosome has not undergone mutation. This indicates that a V gene sequence alone is not sufficient for somatic mutation to take place. The mutation is therefore restricted to the rearranged V gene and consequently does not occur before rearrangement.*

Immunoglobulin genes are striking in their sequence diversity and in the DNA rearrangements associated with their expression. The productive rearrangement required for light chain expression involves the positioning of one of the several hundred variable (V) genes next to the joining (J) region of the constant (C) gene. For heavy chain expression, two rearrangements are necessary since a third region, the diversity (D) region, is incorporated in the product. Usually, only one immunoglobulin molecule is produced per cell, a phenomenon called allelic exclusion. The exclusion of one of the two alleles results from either the absence of a V-J rearrangement or from an aberrant form of rearrangement.

The diversity of immunoglobulin genes results from (i) the existence of a large number of different V genes and a smaller set of D and J regions in germline DNA, (ii) variability introduced at a limited number of sites in the process of V-J or V-D joining (or both), and (iii) the occurrence of somatic mutations affecting V genes. Somatic mutation of immunoglobulin V genes was originally described for V lambda genes (*1*) and is now well established for V kappa (*2–4*) and V heavy genes (*5–8*). However, the nature of the mechanism responsible for these V gene–specific somatic mutations and the extent of its action are still not understood (*9*). Since there are several hundred different V genes in the mouse genome, an important question concerning the mutation of these genes is: are most or all V genes mutated because the mechanism responsible for mutation recognizes V gene sequences in a differentiated lymphocyte or are mutations limited to the V genes that have undergone V-J joining?

If somatic mutations result from a generalized mechanism in differentiated lymphocytes, based on the recognition of V gene sequences, then most or all V genes should be found mutated when compared with their germ-line homologs. However, if mutations are restricted to the site of V-J joining, then only the particular V gene involved in the rearrangement would be subject to mutations. An unambiguous distinction between these alternatives can be obtained by a DNA sequence analysis of the same gene, rearranged and mutated on one chromosome and not rearranged on the other chromosome. The V kappa light chain genes from mouse myeloma T (Fig. 1) are well suited for such a study because in this myeloma (i) V-J joining has taken place on both chromosomes (*10, 11*), thus excluding any possible difference in susceptibility between V genes on a rearranged and on a nonrearranged chromosome; (ii) on each chromosome the V gene involved in the V-J rearrangement has undergone somatic mutation (*2*), showing that the mutation mechanism has operated on both chro-

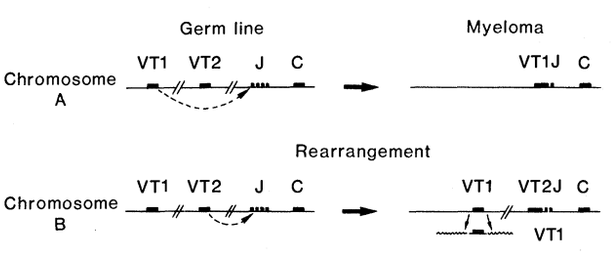

Fig. 1. Schematic description of myeloma T. Rearrangement has taken place on both chromosomes (*8, 9*). On chromosome A, V gene T1 has been joined to J1. On chromosome B, V gene T2 has been joined to J2; however, a copy of VT1 is still present. This V gene was cloned in phage and sequenced. Both V genes T1 and T2, which have undergone rearrangement, have been shown to have undergone somatic mutation (*2*).

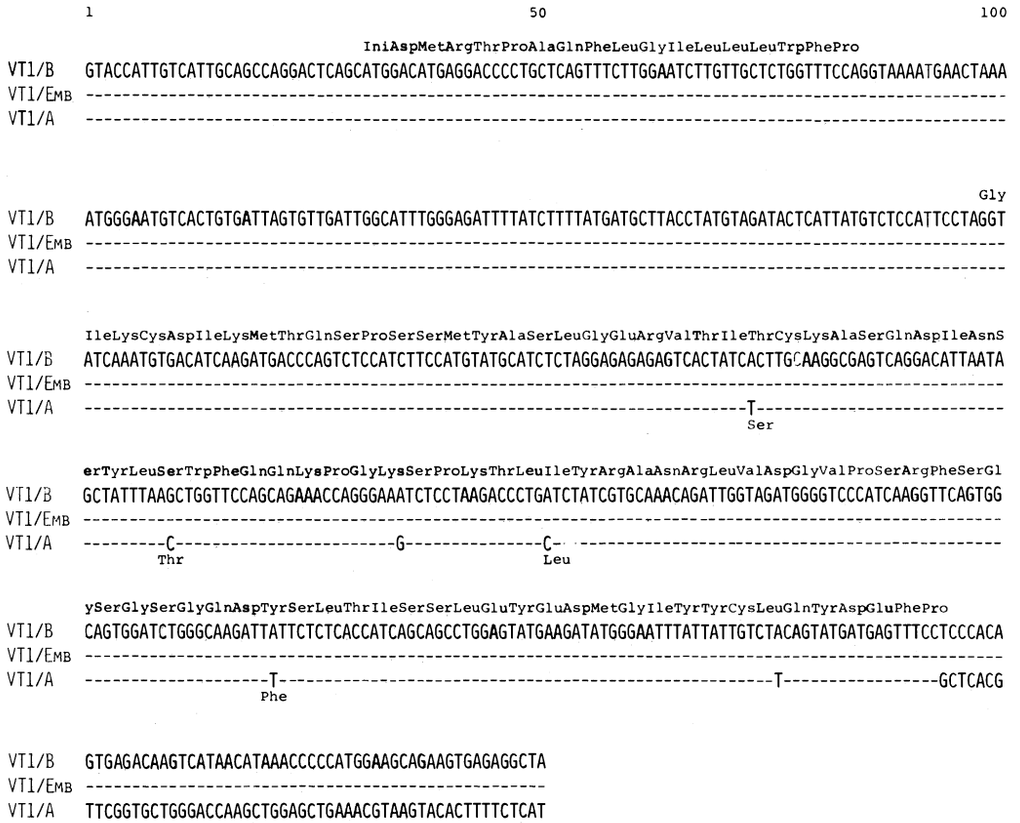

Fig. 2. Sequence of VT1 from chromosome B compared with the same V gene from the germ line, VT1/EMB. The sequence for VT1/EMB is taken from Pech *et al.* (*2*). Also included in this comparison is the sequence of VT1 rearranged, VT1/A (*15, 16*). Dashes indicate identity.

mosomes; and (iii) one V kappa light chain gene (VT1), which is rearranged (and mutated) on one chromosome (A), is still present on the other chromosome (B) obviously not joined to a J region (Fig. 1). We could distinguish between the two models mentioned above by determining whether or not that same V kappa light chain gene on chromosome B has undergone somatic mutation.

We have cloned the V kappa gene VT1 from chromosome B. The same gene that has undergone rearrangement and mutation on chromosome A has been cloned (*10*). In germ-line DNA, this VT1 gene is found on a 3.5-kilobase (kb) Eco RI fragment (*11*); the Southern hybridization technique showed that this fragment is conserved in myeloma T (data not shown) (*10*). The DNA from myeloma T was digested with Eco RI and enriched for DNA less than 10 kb in length. This DNA was ligated to lambda Wes gt, packaged in vitro, and introduced into *Escherichia coli* K803. Clones hybridizing with a ^{32}P-labeled DNA probe specific for the VT1 gene family (*12*) were identified by filter hybridization and analyzed by gel electrophoresis. Those containing the 3.5-kb insert (two isolates) were used for subcloning in plasmid pBR322. A 1.5-kb Eco RI–Hind II fragment containing the V gene was prepared, and fragments generated from it were subcloned in bacteriophage M13. The sequence of the VT1 gene was determined by the dideoxy sequencing method (*13, 14*).

We compared the sequence of the VT1 gene from chromosome B of myeloma T to that of the rearranged VT1 gene from chromosome A (*15, 16*) and to that of the VT1 gene from germ-line DNA (*2*). The VT1 gene from chromosome B is identical to that of the germ-line (VT1/EMB) DNA (Fig. 2) and, as reported previously (*2*), the VT1 gene from chromosome A (VT1/A) has undergone six point mutations. The identity of the VT1/B sequence with that of VT1/EMB shows that this gene has not undergone somatic mutation.

This observation indicates that somatic mutation of V genes is not a general developmental phenomenon affecting most or all V genes in a differentiated lymphocyte but rather that it is restricted to the V gene at the site of V-J joining. Although in this case we are generalizing from one observation, the system studied here, in which V-J joining and somatic mutation have taken place on both chromosomes, optimizes the chances of detecting possible mutations in a V gene not directly involved in rearrangement.

The following conclusions can be drawn about the somatic mutation of V genes. First, there are cases in which it does not occur (*17*), and second, when it does occur, it is restricted to the V gene at the V-J joining site. Consequently somatic mutation does not occur before the rearrangement of a V gene. Somatic mutation could take place during the rearrangement process or could be a consequence of the rearrangement. The former possibility has been suggested for kappa light chain genes (*3*). For heavy chains, the latter possibility is suggested on the basis of a preferential mutation of genes that have undergone isotypic switch (*5, 7, 8*) and thus have had time to accumulate these mutations. If mutation is indeed a consequence of rearrangement, the DNA segment recognized by the mutation mechanism is either (i) the sequence newly created by the V-J joining, (ii) the J region sequence itself, independent of the presence of a V gene (which can be tested by sequencing the J region of a nonrearranged plasma cell chromosome), or (iii) the V gene sequence itself, but now recognized only because it is being transcribed and thus is in a different conformation.

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Clot-Selective Coronary Thrombolysis with Tissue-Type Plasminogen Activator

Abstract. *Coronary thrombolysis, an intervention that can abort the sequelae of acute myocardial infarction, was accomplished within 10 minutes in dogs by intravenous administration of clot-selective, tissue-type plasminogen activator. In addition to inducing clot lysis, this promising fibrinolytic agent restored intermediary metabolism and nutritional myocardial blood flow, detectable noninvasively with positron tomography, without inducing a systemic fibrinolytic state.*

Early coronary thrombolysis is being evaluated extensively as a means to abort the sequelae of acute myocardial infarction (*1*). However, its efficacy is difficult to assess and the procedure generally used requires cardiac catheterization (*2*). Intracoronary administration of activators of the fibrinolytic system such as streptokinase and urokinase induce proteolysis in the circulating blood and can induce a lytic state that may lead to systemic bleeding (*3*). Thus, this approach is not without risk and entails unavoidable delay that may limit the efficacy of the intervention. The interval after acute thrombosis during which reperfusion is salutary is clearly limited (*2, 4*) and the necessity for cardiac catheterization contributes to additional delay.

Physiologically, fibrinolysis occurs only at the thrombus, where the binding of tissue-type plasminogen activator (tPA) to fibrin results in a complex with high affinity for circulating plasminogen (*5, 6*). Plasmin, produced at the fibrin surface, is protected from interaction with α_2-antiplasmin because its lysine binding sites are occupied. Excess free plasmin forms complexes with circulating α_2-antiplasmin through unoccupied lysine binding sites and is thus inactivated (*5*).

We attempted to determine whether selective coronary thrombolysis could be achieved without induction of a systemic lytic state by intravenous administration of an agent with properties similar to those exhibited by the circulating, physiological activator and whether favorable effects on metabolism of previously ischemic myocardium could be elicited. Accordingly, tPA was purified from the culture medium of a melanoma cell line and administered to dogs with induced coronary thrombi. Myocardial perfusion and metabolic integrity were evaluated noninvasively with positron emission tomography (PET) before and after administration of the tPA.

Coronary thrombolysis can be detected unambiguously by serial, coronary arteriography, but objective assessment of the metabolic and functional consequences of myocardium previously rendered ischemic is difficult. Relief of chest pain, rapid resolution of electrocardiographic ST-segment deviations, increases in left ventricular ejection fraction, and diminution of defects in thallium-201 scintigrams have been used as criteria of benefit in patients but are difficult to interpret (*2*). To characterize myocardial perfusion and the metabolic response to reperfusion, we used an approach applicable to patients as well as to experimental animals. Dogs were studied by PET after intravenous administration of ^{15}O-labeled water and ^{11}C-labeled palmitate (*2, 4, 7, 8*).

Twenty-four dogs (20 to 26 kg) were injected subcutaneously with morphine sulfate (1.0 mg/kg) and anesthetized with sodium thiopental (12.5 mg/kg) and α-chloralose (60 mg/kg) administered intravenously. Coronary thrombus was induced by advancing a copper coil, under fluoroscopic control, into the left anterior descending coronary artery (*4, 9*). Occlusive thrombus, heralded by typical electrocardiographic signs of ischemia, was confirmed angiographically. Dogs that developed ventricular fibrillation after coil placement and coronary thrombosis ($N = 7$) were excluded.

One to two hours after induction of coronary thrombus, the dogs were selected at random to receive intracoronary (i.c.) or intravenous (i.v.) streptokinase or tPA. Doses of streptokinase for both routes were 4000 IU/min ($N = 8$ i.c. and $N = 6$ i.v.) and were given for 90 minutes or longer if thrombolysis was not achieved. Although tPA has high specific fibrinolytic activity, its half-life ($t_{1/2}$) is short (2 to 3 minutes) (*10*). Accordingly, and because we wished to determine whether high dosage would be

Table 1. Effect of streptokinase (SK) and tissue-type plasminogen activator (tPA) in dogs with coronary thrombosis. The tPA was given by the i.c. route to five dogs and by the i.v. route to another five dogs. The SK (4000 U/min) was given to eight dogs by the i.c. route and to six dogs by i.v. administration. All values are expressed as means ± standard error. Differences between groups were assessed with the use of the Mann-Whitney *U* test; FDP, fibrinogen degradation product.

Agent	Route of administration	Time of onset of lysis (min)	Percentage change in tomographically estimated jeopardized myocardium*	Percentage change after thrombolysis compared to prethrombolytic values†			
				Fibrinogen‡	FDP	Plasminogen	α_2-Antiplasmin
tPA	i.c.	8 ± 1§	−57 ± 18§	−10 ± 7	6 ± 5§	8 ± 17§	20 ± 10§
tPA	i.v.	8 ± 1§					
SK	i.c.	31 ± 2§	−25 ± 12§	1 ± 7	33 ± 18§	−12 ± 14§	−10 ± 10§
SK	i.v.	85 ± 19§					

*The percent salvage of myocardial metabolism assessed after [^{11}C]palmitate administration was calculated as previously described (*4, 15*). †Values were expressed as percentages of the levels before thrombolytic therapy in order to normalize for variation from animal to animal. ‡Fibrinogen depletion is not anticipated with the dose of SK selected because of two factors: (i) SK binds less avidly to canine than human plasminogen; and (ii) the SK-plasmin complex does not activate plasminogen as efficiently in dogs as in humans (*13*). §$P < .05$.