in three additional *sevenless* alleles (*3*).

In an earlier study (*5*), it was suggested that the presumptive R7 cell was absent from the developing *sevenless* ommatidium. This interpretation may have been based on the appearance of stages equivalent to that represented in Fig. 2D, when the transformation has already taken place.

Drosophila eye cells are directed into their developmental pathways by environmental cues rather than by cell lineage (*6, 9*), and a cell occupying the R7 niche is normally fated to become R7. It is not known whether a mutant cell attempts to become R7 but defaults into the cone cell pathway or whether the mutation specifically orders an uncommitted cell into the cone cell pathway. The cell in the R7 niche displays the characteristic dynamics of R7 until comparatively late in the nuclear choreography, but as early as the symmetrical cluster stage the absence of an axon indicates it has deviated from the photoreceptor pathway.

The cell occupying the R7 niche in *sevenless* appears to be developmentally misdirected by an intrinsic defect rather than by an abnormal cellular environment. Mosaic eyes composed of a mixture of normal and *sevenless* cells (marked with the pigment-deleting mutation *white*) do not contain *white* R7 cells (*4, 5*); normal surrounding cells are unable to rescue a mutant cell in the R7 position. Conversely, pigmented R7 cells can be found in otherwise *white*, and thus genetically *sevenless*, ommatidia, indicating that surrounding mutant cells cannot pull a normal R7 cell into mutant behavior (*4, 5*). The cell in the R7 niche thus appears to receive the correct developmental cues but is unable to respond appropriately. This cell-autonomous action of *sevenless* is distinct from the "transfating" observed in the leech and nematode, in which a changed cellular environment can cause a cell to switch its fate (*10, 11*).

In the mutant, an "extra" nucleus does not emerge from the basal pool and display recognizable cone cell behavior. Thus we cannot yet tell if a redundant cone cell is never elicited in the mutant retinal epithelium or if a presumptive cone cell is developmentally redirected or dies. Cell death normally removes supernumerary cells from the developing retina, and in *sevenless* degenerating cells are often observed near the site where the usurped cone cell normally rises.

Mutations effecting cell-specific transformations have also been described in *Drosophila* and in the nematode *Caenorhabditis elegans*. In *Drosophila*, the mutation *Hairless* causes cells normally fated to produce bristles to become socket-secreting cells instead (*12*). Mutations in the nematode gene *lin-12* can cause cells that normally follow divergent developmental pathways to adopt identical fates, leading to the duplication of certain structures and the absence of others (*13*).

When mutations act embryologically in *Drosophila*, large areas of tissue become developmentally transformed. Operating at the end of the determinative events in the fly's retina, *sevenless* causes only a single cell to switch its fate. Such an aberration occurring in this well-characterized cellular milieu offers an excellent opportunity to examine the machinery of a developmental decision.

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Dynamics of Lymphocyte-Endothelial Interactions in Vivo

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The dynamics of the attachment of lymphocytes to the endothelium of high endothelial venules in murine Peyer's patches were studied in vivo. Lymphocytes adhered readily to the endothelium lining these vessels, but most of the adhering cells detached within a few seconds. Many lymphocytes, however, experienced multiple collisions with the high endothelial venules, and this substantially increased the efficiency of lymphocyte collection by these vessels.

LYMPHOID TISSUES SUCH AS PEYER'S patches and lymph nodes routinely extract large numbers of blood-borne lymphocytes. The postcapillary venules of these lymphoid tissues have a specialized high endothelium to which lymphocytes adhere and then penetrate (*1*). For this to occur, a series of events must take place. First, the lymphocyte must arrive in the high endothelial venules (HEV) by way of the bloodstream and then collide with the endothelium. During this collision, the lymphocyte must adhere to the endothelium with sufficient tenacity to prevent it from being dislodged by hemodynamic forces. Furthermore, this attachment must occur at a site that permits penetration. Although the chance of a lymphocyte accomplishing all this would appear to be low, this is clearly not so.

Many specialized aspects of the anatomic and molecular basis of this unusual lymphocyte-endothelium interaction are understood (*2–13*). The dynamics of the process, however, have not been examined. We studied the interaction of fluorescently labeled lymphocytes with the endothelium of postcapillary venules of Peyer's patches in vivo, and we examined the dynamics of the lymphocyte attachment-detachment and collection processes in the HEV.

Peyer's patches bearing jejunal loops were exteriorized from female BALB/c mice anesthetized with pentobarbital (60 mg/kg). Postcapillary venules adjacent to follicles of the patches (Fig. 1) were visualized with an epifluorescence-equipped microscope. Single-cell suspensions of syngeneic mesenteric lymph-node lymphocytes were labeled with tetramethylrhodamine isothiocyanate

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(RITC); 4 μg of RITC was used per 5×10^7 cells for each milliliter of RPMI 1640 medium containing 5 percent fetal bovine serum (FBS). Labeling under these conditions has no detectable effect on lymphocyte viability or in vivo migration (*14*). The labeled lymphocytes were immediately centrifuged through FBS, washed and resuspended in RPMI 1640, and injected into recipient animals through a lateral tail vein (2×10^6 cells in 0.2 ml of RPMI). Video recordings were made with a silicon-intensified target video camera. We studied the cells that arrived within the first 30 seconds after the appearance of the first cell (1 to 3 seconds after injection). The video recordings permitted a temporal analysis (60 frames per second) of labeled lymphocyte-endothelial interactions.

Labeled lymphocytes entered the HEV at high speed, collided with the endothelium, and came to a complete halt in less than 1/30 second after collision. The HEV were lined with fluorescing cells within minutes. In contrast, other vessels and those serving adjacent nonlymphoid intestine were essentially devoid of adhering cells.

We called an adhesion that lasted two frames (that is 1/30 second) or more a "hit" and determined the number of frames each hit persisted (Fig. 2). Most lymphocytes experienced a hit, but a majority of these quickly detached (Figs. 2 and 3). Most detachments occurred within the first few seconds. None were observed after 12 seconds, and thus hits that lasted more than 30 seconds were designated "secure."

Initial attachment of lymphocytes to the HEV appears to be mediated primarily by binding of complementary surface components of lymphocytes and high endothelial cells (*8–13*). In theory, for a lymphocyte to adhere, the number of bonds formed during a collision (perhaps as few as one) must be sufficient to hold the lymphocyte in the presence of hemodynamic forces (*15*). But the number of effective bonds or the energy in the system probably fluctuates because of thermal and other effects. If the number of bonds reaches an approximate steady state by the time a cell has adhered for 1/30 second (*16*), and the rate of fluctuation is finite, the theory of extremes (*17*) implies that the process describing the time until the number of bonds first crosses the critical level (that is, the time to detachment) would be asymptotically exponentially distributed. Thus, the time to detachment of the ith lymphocyte would be a random variable with an approximately exponential distribution having a mean attachment time $1/\mu_i$. The detachment process may depend on both the lymphocyte and the attachment site. For example, the population of lymphocytes we used consisted of roughly equal numbers of B and T cells (*18*), and it is possible that Peyer's patch HEV-binding properties differ among types of lymphocytes (*6–13*). Similarly, the geometry of the interaction site might influence the decay rate of an attached cell through local differences in hemodynamic forces, distribution of endothelial surface molecules, or the degree of contact. The precise details are not known, but it seems reasonable to suggest that specific lymphocyte-endothelial cell interactions may be characterized by exponentially distributed detachment times with a

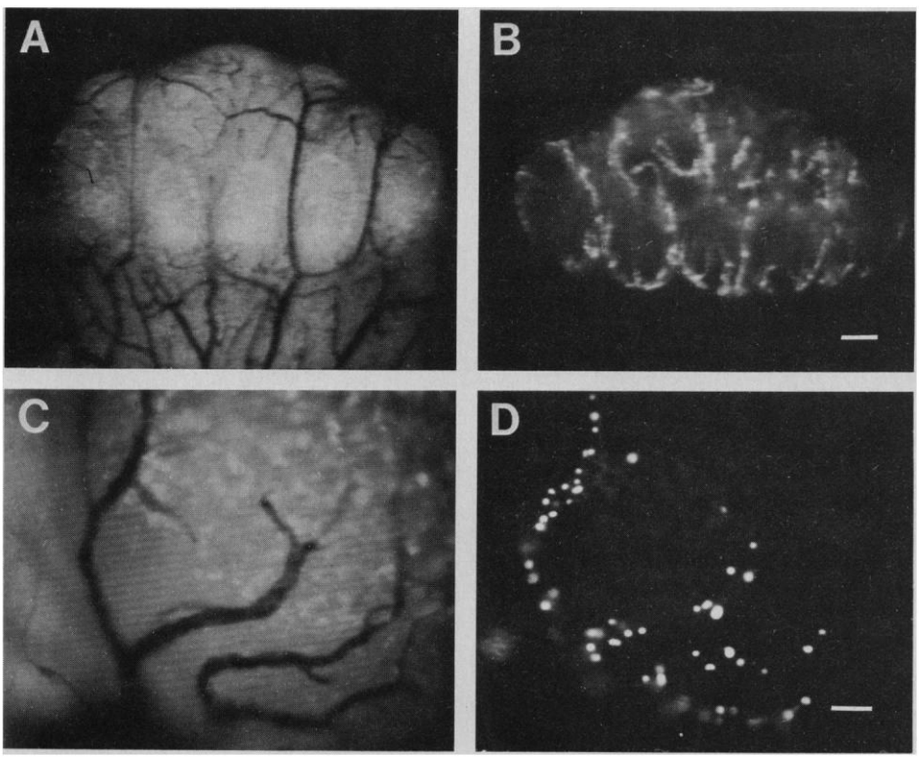

Fig. 1. Photomicrographs of the collection of injected fluorescently labeled lymphocytes by the endothelium of postcapillary venules (HEV) of mouse Peyer's patches. (A) Autofluorescence under blue light, showing vessels lying between follicles. (B) Same as (A) but under green excitation, showing rhodamine-labeled lymphocytes (1 hour after injection) accumulating in vessels between follicles, but not in adjacent nonlymphoid intestine (scale bar, 300 μm). (C) View of HEV (blue light autofluorescence). (D) Same as (C) but under green excitation showing labeled lymphocytes adhering to HEV 10 minutes after injection (scale bar, 50 μm).

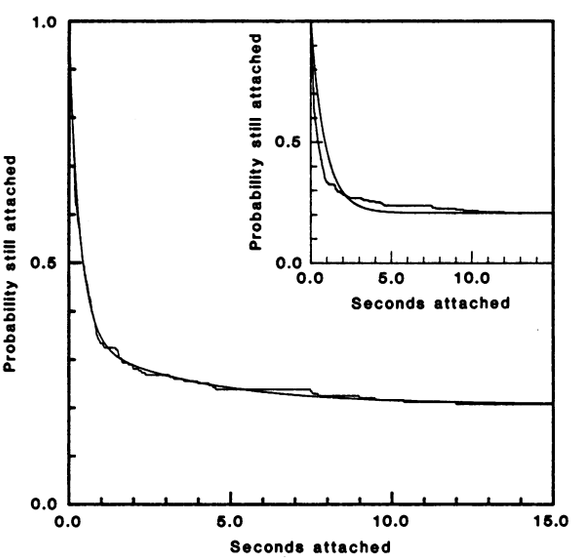

Fig. 2. Probability that a lymphocyte remains attached to the HEV at time t after attachment. Main figure shows the data for 183 detaching and 48 secure hits pooled from three animals and a maximum likelihood fit of Eq. 2. The insert shows the same data and a fit of Eq. 1 with two terms (see text).

range of decay rates. Thus, the probability that a lymphocyte remains attached after time t would have the form

$$P(t) = \sum_i b_i \exp(-\mu_i t) \quad (1)$$

where b_i is the proportion of lymphocyte-endothelial detachments with mean detachment time $1/\mu_i$.

Not all attached lymphocytes, however, detach (Figs. 2 and 3). Those lymphocytes that hit penetrable areas between adjacent endothelial cells and that remain attached there long enough can enter the patch by insinuating between endothelial cells (*2–4*). Some of the lymphocytes observed (Fig. 2) remained attached for the duration of the experiment. A simple view of these cells is that their mean time to detachment is very long or effectively infinite. Maximum likelihood fits of Eq. 1 under the assumption of two differing classes of attachments, one with infinite detachment time and one with finite detachment rate μ, are shown in Fig. 2. The fit of the equation with two terms is in qualitative agreement with the data, but in detail the fit is poor and is rejected by the χ^2 test ($P < 0.001$; d.f., 12). With three terms in the series, however, a good fit ($P > 0.8$; d.f., 12) is obtained, and Eq. 1 becomes

$$P(t) = 0.21 + 0.14 \exp(-0.29t) + 0.65 \exp(-3.0t) \quad (2)$$

Satisfactory fits could also be obtained with any larger number of terms in Eq. 1, or with other models.

There are a number of possible explanations for the failure of the single exponential form of Eq. 1. For example, the fit of Eq. 2 suggests the possibility that there may be more than two classes of lymphocyte-endothelial interactions. Alternatively, our assumption of an approximate equilibrium of the binding process may not be entirely valid, or the critical binding level required for sustained lymphocyte attachment and the equilibrium level of binding may be close enough to each other that the asymptotic exponential form is not a good approximation.

Within the limits of the present experimental data, the heterogeneity of attachment can be adequately accounted for by a simple model in which about two-thirds of the lymphocytes detach quickly, with a mean time to detachment of about 0.3 seconds; about one-seventh have a mean time to detachment of about 3.4 seconds; and the rest persist and probably eventually penetrate the HEV.

Another feature of the interaction of lymphocytes and the HEV is the pinball-like nature of the collection process. Many hits are temporary; many cells hit more than once; and some cells never hit on their way through (Fig. 3). The cells behave as if there is a constant probability p that a free cell will hit and a probability c that a hit is secure. We propose a simple model in which a detached cell has probability p of hitting again and probability c of becoming permanent if it does hit (Fig. 3). Thus, the probability q that a cell entering a HEV will be secure is

$$q = pc + p(1 - c)pc + p(1 - c)p(1 - c)pc + \ldots$$

$$= pc \sum_{k=0}^{\infty} [p(1 - c)]^k$$

$$= pc/[1 - p(1 - c)] \quad (3)$$

Maximum likelihood estimates of the parameters were $p = 0.63 \pm 0.05$ (± 95 percent confidence limits), $c = 0.21 \pm 0.05$, and $q = 0.26 \pm 0.06$.

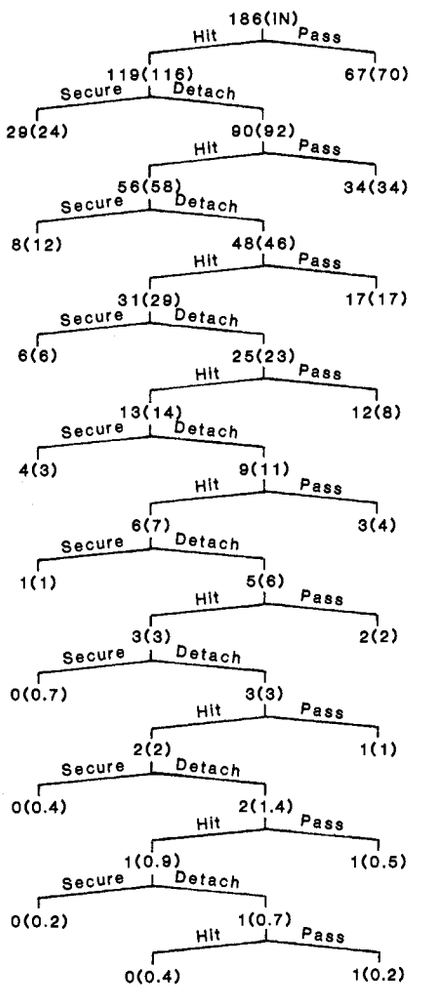

Fig. 3. Pattern of the multiple collision process of lymphocytes in the HEV, illustrating the recurrent nature of the process for lymphocytes experiencing multiple collisions. At the top, 186 cells enter the HEV. Of these, 67 were observed to pass directly out of the vessel. Of the remaining 119, 29 became secure and 90 detached. These 90 detached cells then repeated the process. The numbers in parentheses are those expected from the model (Eq. 3).

Several features of the multiple collision process merit emphasis. First, most cells (63 percent) that enter the postcapillary venules of Peyer's patches collide with the endothelial surface, but most of the collisions (79 percent) lead to only temporary adhesion. Nevertheless, the overall efficiency of the collection process is quite high (26 percent), since the multiple collisions experienced by many cells effectively amplifies the probability of a secure hit. Second, if lymphocytes act independently of one another (that is, the cell delivery rate is low so that adherence of a cell does not materially affect adherence of another cell), the probability that m out of n cells entering a draining venule will attach securely would follow a binomial distribution with mean nq and variance $nq(1 - q)$. Consequently, the collection process for large numbers of lymphocytes entering a large number of vessels could be adequately modeled as a first-order deterministic process (*19*). In this regard, the value we obtained for q is consistent with estimates derived by less direct means for the proportion of blood-borne lymphocytes that gain entry to Peyer's patches (*19*).

We have directly examined in vivo the dynamics of the interaction of lymphocytes with the endothelium of postcapillary venules. This approach permits a quantitative assessment of the speed, extent, and efficiency of lymphocyte-endothelial cell interactions. Further studies of the dynamic behavior of selected lymphocyte populations and the effects of various agents or manipulations of cell surface components on this behavior is now possible and may offer new insights into the nature of the processes governing the interaction of lymphocytes and these specialized endothelial cells.

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Reductions in Arterial Diameter Produced by Chronic Decreases in Blood Flow Are Endothelium-Dependent

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A 70 percent reduction in the rate of blood flow through the common carotid artery in rabbits caused a 21 percent decrease in the diameter of this artery within 2 weeks. The smooth muscle relaxant papaverine did not attenuate the response; therefore, such reductions in diameter probably reflect a structural modification of the arterial wall rather than sustained contraction of smooth muscle. This arterial response to reduced blood flow was abolished when the endothelium was removed from the vessels. It appears that the endothelium is essential for the compensatory arterial response to long-term changes in luminal blood flow rates.

THE INNER DIAMETERS OF MAMMALIAN arteries are correlated with luminal blood flow rates throughout development (*1*). This dependence of arterial size on blood flow is not totally preprogrammed in vascular development, however, because experimental interventions that alter blood flow cause corresponding changes in arterial diameter (*2, 3*). Thus, local hemodynamic forces are important in determining vascular structure. This responsiveness to local blood flow persists into adulthood (*4*) and may play a role in arterial occlusive disease. For example, flow reductions due to partial occlusion may be exacerbated by constrictor responses to the reduced flow. On the other hand, early increases in flow through collateral vessels after arterial occlusion may be enhanced by a dilatory response to the increased flow.

Flow-induced changes in arterial size are mediated locally because they are restricted to the sites at which flow is altered, they are not abolished by arterial denervation, and they are not dependent on coincident changes in arterial pressure (*2, 5*); however, little else is known about the mechanisms underlying this important response. Changes in arterial size, whether contractile or structural, must be effected by vascular smooth muscle cells, since these are the only cells in the media of adult arteries, but it is unlikely that these cells directly sense changes in blood flow rates through the vessel lumen. Such direct sensitivity to flow would imply that the cells detect changes in shear stress; however, physiological shear stresses cause extremely little bulk deformation of the arterial wall. For example, 100 dynes/cm$_2$, a very high shear stress in vivo (*6*), will cause less than 1 percent bulk deformation (shear strain) of the arterial wall tissue (*7*). Therefore, it is unlikely that cells deep in the vessel wall can detect changes in blood flow.

Instead, we and others (*5, 8*) have reasoned that the endothelium, which is in direct contact with luminal blood, may detect changes in flow and mediate responses of the smooth muscle. The endothelium may release vasoactive substances (*9*) that adjust smooth muscle tone and vessel wall growth modulators (*10*) that initiate structural modifications of the vessel wall. There is evidence that the release of some of these agents is dependent on shear stress (*9*). In the study reported here, we altered blood flow rates through the one common carotid artery of rabbits and determined the influence of endothelial denudation on inner arterial diameter.

Adult male New Zealand White rabbits were anesthetized intramuscularly with ketamine (22 mg/kg) and xylazine (2.2 mg/kg) and the left external carotid artery was exposed and ligated at its origin. Therefore, flow through the left common carotid artery was reduced to internal carotid artery flow. In seven of these rabbits, electromagnetic flowmeters were implanted on either the left (four rabbits) or right (three rabbits) common carotid artery 1 week before surgery, and blood flows were recorded until 1 week after surgery. Ligation of the left external carotid caused a 70 percent reduction in blood flow, from 26.5 ± 3.00 to 8.0 ± 0.70 ml/min (means ± standard errors), and no significant change in flow through the contralateral common carotid.

The rabbits were killed by barbiturate overdose 2 weeks after surgery and a methacrylate casting compound (Batson's No. 17) was immediated infused into the carotids via the thoracic aorta under physiological pressure (100 mmHg) and allowed to set for 30 to 60 minutes. The rigid casts, with arterial tissue, then were removed and immersed in a fixative. The next day the tissue was removed from the cast and cross-sec-

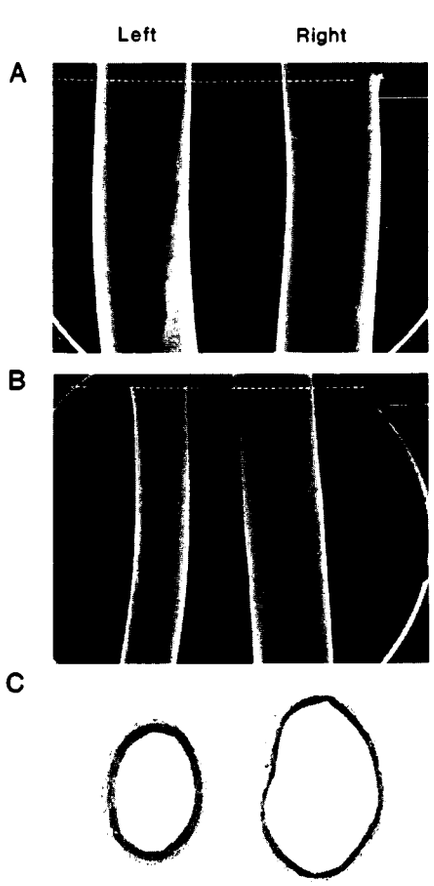

Fig. 1. (A) Scanning electron micrograph of methacrylate casts of the right and left common carotid arteries of a normal rabbit. (B) Micrograph of carotid casts 2 weeks after left carotid flow was reduced. Markers are 100 μm. (C) Histological cross sections of carotid arteries 2 weeks after left carotid flow was reduced.

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