INFLAMMATION 1987—THE OLD AND THE NEW

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It is a great privilege to give this lecture, and I only wish I could give it in Japanese.

Inflammation can be defined as a reaction typical of vascularized tissues, whereby fluid and cells, carried by the blood, accumulate at a site of local injury. This is the traditional definition. However, in 1987 it becomes necessary to add that according to recent discoveries *inflammation is no longer the only response of tissues to injury*. There are in fact two separate, independent responses. One is inflammation, which—as I just mentioned—will cause cells and fluid to accumulate at the site of injury; the other is an *intracellular* response, which caused injured cells to accumulate a special set of proteins, which have been called heat-shock proteins or simply shock proteins^{1,2}. Their discovery was triggered by observations of an Italian biologist, Ritossa, in 1967. In studying larvae of the fruit fly (*Drosophila*) Ritossa found that if he submitted the larvae to a mild increase in temperature for as little as 30 minutes its chromosomes showed localized swellings (“puffs”) which are now known to be places of accelerated RNA synthesis, and thus indicate increased protein synthesis. During the past few years it was discovered that this is a general phenomenon: the synthesis of new, specific intracellular proteins after injury can be observed in all animals from man down to bacteria, in tumors as well as in plants, and not only after heating but after any kind of insult. The so-called shock proteins have been sequenced and found to be very similar from bacteria to man, which means that in evolution they must have played a very important role. Their precise function, however, is still a mystery. Some appear to concentrate on the nucleolus.

This field of study is expanding rapidly. I decided to mention it here because future meetings like ours today may well need to include a section on the Shock Proteins.

Now let us turn to the inflammatory reaction. This lecture will focus on the pathophysiology of vascular leakage: a topic of theoretical as well as practical importance, because it is obvious that any pharmacologic intervention aimed at reducing the inflammatory swelling must be targeted to specific microvascular mechanisms.

At least two mechanisms of vascular leakage are involved: (1) *direct non-specific injury* to the blood vessels; (2) *functional responses* of the endothelium to chemical factors from damaged tissues. All types of microscopic vessels can be affected: arterioles, capillaries and venules. In order to understand which section of the microcirculation has become abnormally permeable (leaky), histology alone is of little help. Therefore we use a very simple method based on the following principle: in the microcirculation, the lumen is surrounded by two principal

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Fig. 1 scheme of vascular labeling in the microcirculation

Wherever a gap appears in the endothelial layer (while the basement membrane remains intact) plasma leaks out (fine dots); colloidal particles carried by the blood stream accumulate against the basement membrane and form a deposit (larger dots). If the particles represent a pigment (injected intravenously) they can form a deposit that can be seen by electron microscopy, by light microscopy, and even by the naked eye.

layers: the endothelium, which represents the permeability barrier; and the basement membrane, shaped essentially like a sleeve around the vessel. If a microscopic vessel is injured, very often the endothelial layer is interrupted, but the basement membrane remains intact. In an experimental animal, we can demonstrate that such a vessel has become leaky by injecting into the blood stream a colloidal suspension of particles too large to traverse the basement membrane (such as carbon black). At the site of the leaks, plasma will escape; however, the particles of carbon black will be retained by the basement membrane, and accumulate against it, like coffee on a filter (Fig.1). These deposits are large enough to be easily seen with the light microscope and even with the naked eye³⁾. The pigment Monastral Blue B was also proposed for the same purpose⁴⁾.

If either one of these pigments is injected into the blood stream of an animal which has suffered a local injury, leaky vessels in and around the injury will become "labeled" with the pigment; if the tissues are then excised, fixed, and made transparent with glycerin, all the leaky vessels will be either black or blue, and it will be easy to classify them (by their arrangement) as arterioles, capillaries or venules.

Using this method we found that *direct injury*, as predicted, causes all the microscopic vessels to leak (arterioles, capillaries, and venules). Obviously, no type of microscopic vessel can escape direct injury.

Venular leakage The result is very different when inflammatory mediators are applied. In 1961 we tested three mediators of inflammation known at that time (histamine, serotonin, bradykinin) and found that they caused leakage to occur selectively from the venules, whereas the capillaries remained intact. We called this "histamine-type leakage". The mechanism was explained later: it depends on contraction of endothelial cells in the venules⁵⁾. This was confirmed by observations on the living vessels by Joris et al⁶⁾. The question, then, remained: why should the venules alone respond to the mediators? An answer was provided by a Romanian group⁷⁾: receptors for histamine are much more numerous in the venules than in the other segments of the microcirculation.

Fig. 2a venule in guinea-pig abdominal muscle 11 minutes after an injection of LTE_4 (830 ng) and intravenous injection of carbon black. The endothelial cells bulge into the lumen; their nuclei show many indentations. The gap between the two endothelial cells is filled with colloidal carbon. Packed red blood cells in the lumen indicate plasma leakage ($\times 24,200$).

Fig. 2b control venule

The endothelial cells are elongated, flattened and connected by a tight junction; their nuclei have a smooth outline ($\times 18,400$). Courtesy of the American Journal of Pathology⁹⁾

Since the 1960's many new mediators of inflammation have been described: two have been studied by the method of vascular labeling: PAF (platelet activating factor⁸⁾), and leukotriene E_4 ⁹⁾. Both cause the venules to leak selectively, just like the histamine-type mediators; and for leukotriene E_4 it was also shown that the cellular mechanism is again endothelial contraction⁹⁾ (Fig. 2a•2b). To this day, therefore, all the mediators tested cause leakage from the venules only.

Fig. 3 topography of focal arteriolar insudation (FAI)

Drawing from an actual experiment on the cremaster muscle. The oval shaded area represents the place where a blank plastic pellet was implanted; the black segments of the arteriolar tree represent FAI. Note that almost all these segments are at a distance from the pellet. Courtesy of the American Journal of Pathology⁽¹⁾

Fig. 4 FAI, 13 hours after implantation of a histamine-laden pellet

Note accumulation of blood-borne materials in the arteriolar wall; the medial smooth muscle cells have pulled away from one another. There are very few inflammatory cells in the adventitia ($\times 1,800$). Courtesy of the American Journal of Pathology⁽¹⁾

At this point we can predict that leakage from the venules should be preventable by agents that oppose cellular contraction: as you well know, studies by Professor Katori⁽¹⁰⁾ and others have shown that this is indeed the case.

Arteriolar leakage We do not know of any mediator of inflammation that causes arterioles to become leaky. However, in our laboratory, we recently identified a peculiar phenomenon: arterioles can develop small foci of leakage in the vicinity of a focus of inflammation. This can be demonstrated by inserting, in the subcutaneous tissue of a rat, a plastic pellet loaded with histamine—or even a blank pellet: if vascular labeling is performed one day later, leaky segments become apparent in the arterioles that surround the pellet (not in those immediately beneath it) (Fig. 3). The leaky arteriolar segments are always dilated; the endothelium contains gaps, and the smooth muscle cells appear stretched as if paralyzed (Fig. 4). Blood components accumulate in the wall at that point; hence we have given this lesion the name *focal arterial insudation* (FAI)⁽¹¹⁾. At present we have no explanation for FAI; it is certainly not caused by surgical trauma, and it can not be caused by inflammatory cells penetrating into the arterial wall, because we have found no such infiltrate. Similar arteriolar changes have

been obtained by others after perfusion with vasodilators. We can only speculate that FAI may be due to a humoral or neural effect originating from the inflammatory focus. We believe that FAI may be significant for understanding the localization and pathogenesis of various forms of arteritis.

Chronic inflammation: Capillary leakage All the studies of inflammatory mediators performed so far—by means of vascular labeling—were carried out using pure mediators; this type of experiment is of course important, but it does not duplicate the events that occur in tissue injury, in which the inflammatory exudate contains an extremely complex “soup” of mediators. We therefore decided to study vascular leakage caused not by purified mediators but by injured and dying cells. Because dying tissue is known to liberate materials that induce inflammation, we implanted fragments of rat kidney or liver in the subcutaneous tissue or in the peritoneal cavity of another rat, and then studied the effect of the implants on the vessels of the surrounding tissues¹²⁾. Vascular labeling in these animals gave the following results: after 2 to 4 hours the *venules* in the surrounding muscle are labeled, as we had expected; after 12 to 24 hours, *venules as well as capillaries* are labeled; at 48 hours the leakage comes almost exclusively from the *capillaries*. We are currently seeking an explanation for this specific effect, which is especially interesting because none of the known mediators of inflammation causes capillary leakage.

In summary, we have shown—by means of vascular labeling—that vascular leakage may occur at different stages of the inflammatory response and in different segments of the circulation; venules, arterioles or capillaries. In developing pharmacologic agents to inhibit vascular leakage it may be necessary to consider these separate microvascular targets.

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