Vascular Permeability to Macromolecules Changes Qualitatively in Inflammation ?

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Abstract—The time courses of vascular permeability to native bovine serum albumin (BSA), cationized BSA, dextran (mol wt. 40,000) and bovine immunoglobulin G (IgG) in carrageenin-induced inflammation in rats were determined by the fluorometric method. The vascular permeability to BSA increased gradually until about 5 hr after carrageenin injection. The vascular permeabilities to dextran and IgG reached a maximum at 1 hr after carrageenin injection and then decreased. In the early-stage, 0–1 hr after carrageenin injection, dextran was the most permeating of the three. However, in the later-stage, 3–5 hr after carrageenin injection, BSA became the most permeating. Furthermore, cationized BSA was more permeating than native BSA having a negative charge in the early-stage, but the difference between the permeability to cationized BSA and native BSA was decreased at the later-stage. These data suggest that vascular permeability changes qualitatively in carrageenin-induced inflammation in rats, and it is unlikely that the increased vascular permeability is caused by the ultrafiltration through gaps formed between endothelial cells.

Plasma exudation in inflammatory responses has been generally thought to be due to the formation of gaps between endothelial cells (1–4). Within the limits of this theory, an ultrafiltration mechanism could be applied to the vascular leakage of plasma components. In other words, the permeability of one permeable plasma component in an inflammatory locus would represent the behavior of other permeable plasma components. To estimate vascular permeability to plasma components in an inflammatory locus, some tracers such as dye, radioisotope

or fluorescent probe-labeled albumin or dextran was frequently utilized (5–7). However, in most of the experiments, the investigators used only one tracer and generalize the results as the behavior of other permeable plasma components.

The present study indicates that change in vascular permeability to BSA is not parallel with that to bovine IgG, dextran and cationized BSA and suggest that the vascular permeability changed qualitatively in carrageenin-induced inflammation in rats.

Carrageenin-air-pouch inflammation was induced according to the method described by Fukuhara and Tsurufuji (8). Briefly, male rats of the Sprague-Dawley strain weighing 170–190 g were subcutaneously injected with 8 ml of air on the dorsum to make an air sac, and 24 hr later, 4 ml of 2% (w/v) solution of carrageenin in 0.9% NaCl was injected into the air sac.

Cationization of BSA was performed according to the method of Hoare and Koshland (9). Noncationized and weekly cationized

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Abbreviations: F-BSA, fluorescein isothiocyanate-labeled bovine serum albumin; F-cationized BSA, fluorescein isothiocyanate-labeled cationized BSA; F-IgG, fluorescein isothiocyanate-labeled bovine immunoglobulin G; F-Dextran, fluorescein isothiocyanate-labeled dextran.

BSA were removed by adsorption to DEAE-Sephadex (pH 7.5). Isoelectric point of cationized BSA obtained was 7.5–11.

Conjugations of fluorescein isothiocyanate to bovine serum albumin and bovine immunoglobulin G were performed according to the procedure of McKinney et al. (10) with some modifications. Conjugation of fluorescein isothiocyanate to dextran T-40 (mol wt: 40,000, Pharmacia Fine Chemicals Co.) was performed according to the method of De Belder and Granath (11). Removal of all unreacted fluorescent materials was achieved by washing with cold acetone or ethanol and subsequent dialysis vs. distilled water.

Vascular permeability was measured by the method described in a previous paper (12). In brief, rats were intravenously injected with 0.2 ml of 5% (w/v) tracer-0.9% NaCl solution and sacrificed 15 min after the tracer injection. The entire fluid in the carrageenin-air-pouch was collected, and the volume was measured. One milliliter of pouch fluid was mixed with 5 ml of PBS and then the mixture was centrifuged. When F-cationized BSA was used as tracer, 1% Na_3PO_4 was used instead of PBS to avoid binding of F-cationized BSA to the precipitate. Fluorescence intensity of the supernatant was measured in a fluorescence spectrophotometer (Shimadzu RF-540) with 490 nm for excitation and 521 nm for emission. Total fluorescence intensity in the entire pouch fluid was calculated. Vascular permeability was expressed by Unit values. One Unit was defined as the fluorescence intensity given by same amount of the tracer contained in 1 μl of plasma. One Unit was calculated using the following equation:

$$1 \text{ Unit} = (\text{FI} \cdot 1 + \text{FI} \cdot 15) / 2$$

where,

FI·1=fluorescence intensity of 1 μl of plasma obtained from the blood 1 min after tracer injection

FI·15=fluorescence intensity of 1 μl of plasma obtained from the blood 15 min after tracer injection.

The blood was collected in a heparinized hematocrit tube from the tail artery of rats 1 and 15 min after intravenous injection of the tracer. After centrifugation of the blood in the

hematocrit tube, 25 μl of plasma was exactly measured with a microsyringe.

The time courses of extravasation of F-BSA (effective molecular radius: 36 Å) and F-Dextran (44 Å) are summarized in Fig. 1 A. The extravasation of F-BSA increased gradually until 5 hr after carrageenin injection. On the other hand, the vascular permeability to F-Dextran reached a maximum at 1 hr after carrageenin injection and then decreased gradually. In the early-stage of this inflammation, F-Dextran was more permeating than F-BSA in spite of the fact that it has a bigger effective molecular radius than F-BSA. However, in the later-stage, F-BSA was significantly more permeating than F-Dextran. To eliminate possible influence of each tracer on vascular permeability, unlabeled BSA (10 mg/rat) or dextran (10 mg/rat) was added to the injected solution of F-Dextran or F-BSA, respectively.

The time courses of the change in vascular permeability to F-IgG and F-BSA were determined (Fig. 1B). The pattern of the leakage of F-IgG was very similar to that of F-Dextran (Fig. 1A). Namely, the vascular permeability to F-IgG reached a maximum 1 hr after carrageenin injection. Further, in the early-stage, the vascular permeability to F-

Fig. 1. Time course of changes in vascular permeability to F-BSA, F-Dextran and F-IgG in carrageenin-air-pouch inflammation in rats. F-BSA (10 mg)-dextran (10 mg) or F-Dextran (10 mg)-BSA (10 mg) in 0.2 ml of 0.9% NaCl solution was injected into the tail vein 0, 1, 3 or 5 hr after carrageenin injection in experiment (A). F-BSA (10 mg) or F-IgG (10 mg) in 0.2 ml of 0.9% NaCl solution was injected into the tail vein in experiment (B). Fifteen minutes after the tracer injection, the entire pouch fluid was collected, and vascular permeability was determined. Each point represents the mean $\pm$ S.E.M. of 6–8 determinations.

IgG was almost the same as that to F-BSA, although the effective molecular radius of F-IgG (57 Å) is larger than that of F-BSA (36 Å). On the other hand, in the later-stage, the vascular permeability became less marked to F-IgG than to F-BSA.

F-cationized BSA leaked more markedly than F-BSA having a negative charge under physiological conditions in the period of 0–15 min after carrageenin injection. However, the difference between the permeability to F-cationized BSA and F-BSA was decreased about 5 hr after carrageenin injection (Fig. 2).

The time course of extravasation of F-BSA was different from that of F-Dextran and F-IgG (Fig. 1, A and B). These results indicate that the behavior of a tracer in inflammation does not always represent the behaviors of other tracers. Furthermore, from this fact, it is too dangerous to assume that the permeability change to a tracer will accurately reflect permeability changes to other plasma macromolecules in inflammation.

The most permeating tracers in the early-stage and in the later-stage were F-Dextran and F-BSA, respectively. These data suggest that vascular permeability changed qualitatively in carrageenin-air-pouch inflammation. In the early-stage, there seems to be no restriction on the molecular dimensions of tracers, because the effective molecular radii

of F-BSA, F-Dextran and F-IgG are 36 Å, 44 Å and 57 Å, respectively.

F-BSA, F-IgG and F-Dextran have negative, weakly positive and no charge, respectively, under physiological conditions. Extravasation of these tracers might be influenced more or less by the negative charge of intercellular gaps when these tracers permeate into the inflammatory locus from the intravascular space at the early-stage. In fact, in spite of being the smallest molecule, F-BSA having a negative charge crossed the vessel wall less easily than F-Dextran having no charge, and it was equally as permeable as F-IgG which has a weakly positive charge and the biggest radius.

In renal glomerular filtration, negatively charged macromolecules are restricted by negative charges of the endothelium and basement membrane, while positively charged macromolecules of similar size are filtered in greater amounts (13, 14). However, in nonglomerular capillaries, the negatively charged sites of capillary walls seem to have functions similar to equivalent sites present in the glomerular capillaries (15). The negatively charged sites probably contribute to the low permeability of the microvasculature to negatively charged macromolecules such as albumin. In fact, negative charges locate especially at the clefts leading to the intercellular junctions (16). These negative charges seem to restrict the permeability of negatively charged macromolecules at the early-stage of the carrageenin-induced inflammation. F-cationized BSA was actually more permeating than F-BSA during the first period (0–15 min) (Fig. 2).

On the other hand, this restriction seems to have disappeared at the later-stage, F-BSA having a negative charge was the most permeating of the three tracers. The difference between permeability to F-BSA and F-cationized BSA became small at this stage (Fig. 2). It is presently unclear whether these qualitative changes in vascular permeability were induced by the disappearance or change of the electrical charge around intercellular gaps.

Each tracer may be transported through a different pathway. Casley-Smith and Carter

Fig. 2. Vascular permeability change to F-BSA (F-BSA $\ominus$) and F-cationized BSA (F-BSA $\oplus$). F-BSA (10 mg)-unlabeled cationized BSA (10 mg) or F-cationized BSA (10 mg)-unlabeled BSA (10 mg) in 0.2 ml of 0.9% NaCl solution was injected into the tail vein immediately before or 5 hr after carrageenin injection. Fifteen min after the tracer injection, the entire pouch fluid was collected, and vascular permeability was determined. Each point represents the mean $\pm$ S.E.M. of 6–8 determinations.

(17) suggested that vesicular transport is a pathway to transport plasma components from the intravascular space to the extravascular space in inflammation. Among other possibilities, the difference between binding capacity of each tracer on the pathway from the intravascular space to the extravascular space could have contributed to the changes of tracer level detected in exudate.

Most of the plasma proteins have negative charges under physiological conditions. The qualitative change in vascular permeability that negatively charged molecules become more permeating is probably considered as a response of the host against inflammatory stimuli in order to transport plasma proteins to the inflammatory locus with high efficiency.

The present study suggests that vascular permeability changes qualitatively in carrageenin-air-pouch inflammation in rats and that the increase in vascular permeability is not caused simply by the formation of gaps between endothelial cells and the passage of macromolecules by the ultrafiltration mechanism. More complicated mechanisms seem to be involved.

References

- 1 Majno, G. and Palade, G.E.: Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability. *J. Biophys. Biochem. Cytol.* **11**, 571–605 (1961)
- 2 Majno, G. and Leventhal, M.: Pathogenesis of "histamine-type" vascular leakage. *Lancet* **2**, 99–100 (1967)
- 3 Majno, G., Shea, S.M. and Leventhal, M.: Endothelial contraction induced by histamine-type mediators. *J. Cell Biol.* **42**, 647–672 (1969)
- 4 Cotran, R.S., Suter, E.R. and Majno, G.: The use of colloidal carbon as a tracer for vascular injury. *Vasc. Dis.* **4**, 107–127 (1967)
- 5 Tsurufuji, S., Sugio, K. and Endo, Y.: Inhibition of vascular permeability by cycloheximide in granulomatous inflammation. *Biochem. Pharmacol.* **26**, 1131–1136 (1977)
- 6 Del Maestro, R.F., Björk, J. and Arfors, K.-E.: Increase in microvascular permeability induced by enzymatically generated free radicals. *Microvasc. Res.* **22**, 239–254 (1981)
- 7 Mustard, J.F., Movat, H.Z., Macmorine, D.R.L. and Senyi, A.: Release of permeability factors from the blood platelet. *Proc. Soc. Exp. Biol. Med.* **119**, 988–991 (1965)
- 8 Fukuhara, M. and Tsurufuji, S.: The effect of locally injected antiinflammatory drugs on the carrageenin granuloma in rats. *Biochem. Pharmacol.* **18**, 475–484 (1969)
- 9 Hoare, D.G. and Koshland, D.E., Jr.: A method for the quantitative modification and estimation of carboxylic acid groups in proteins. *J. Biol. Chem.* **242**, 2447–2453 (1967)
- 10 McKinney, R.M., Spillane, J.T. and Pearce, G.W.: Factors affecting the rate of reaction of fluorescein isothiocyanate with serum proteins. *J. Immunol.* **93**, 232–242 (1964)
- 11 De Belder, A.N. and Granath, K.: Preparation and properties of fluorescein-labelled dextrans. *Carbohydr. Res.* **30**, 375–378 (1973)
- 12 Watanabe, K., Nakagawa, H. and Tsurufuji, S.: A new sensitive fluorometric method for measurement of vascular permeability. *J. Pharmacol. Meth.* **11**, 167–176 (1984)
- 13 Chang, R.S.L., Deen, W.M., Robertson, C.R. and Brenner, B.M.: Permeability of the glomerular capillary wall: III. Restricted transport of polyanions. *Kidney Int.* **8**, 212–218 (1975)
- 14 Rennke, H.G. and Venkatachalam, M.A.: Structural determinants of glomerular permselectivity. *Fed. Proc.* **36**, 2619–2626 (1977)
- 15 Vehaskari, V.M., Chang, C.T.-C., Stevens, J.K. and Robson, A.M.: The effects of polycations on vascular permeability in the rats. A proposed role for charge sites. *J. Clin. Invest.* **73**, 1053–1061 (1984)
- 16 Pietra, G.G., Fishman, A.P., Lanken, P.N., Sampson, P. and Hansen-Flaschen, J.: Permeability of pulmonary endothelium to neutral and charged macromolecules. *Ann. N. Y. Acad. Sci.* **401**, 241–247 (1982)
- 17 Casley-Smith, J.R. and Carter, D.B.: The passage of macromolecules across inflamed capillary endothelium via large vacuoles. *Microvasc. Res.* **18**, 319–324 (1979)