Release of Neutrophil and Eosinophil Chemotactic Factor from Sensitized Skin in vitro Cutaneous Anaphylactic Reactions

T.		Numata
K.		Kimura
K.		Tsuboi
S.		Yamada
S.		Yamamoto

Department of Dermatology, Hiroshima University School of Medicine, Hiroshima, Japan

Key Words

NCF
ECF
Chemotactic factor
HPLC LTB₄
Skin, anaphylactic reaction

Tsunemi Numata, MD, Department of Dermatology, Hiroshima University School of Medicine, Kasumi 1-2-3, Minamiku, Hiroshima 734 (Japan)

Histamine is considered to play a critical role in anaphylaxis and produce wheal and flare reactions in skin. On the other hand, it is known that inflammatory cells such as neutrophils and eosinophils migrate into the site of anaphylactic reactions in skin. The cellular infiltrations might produce a prolonged inflammatory reaction after immediate wheal and flare reactions in the cutaneous anaphylaxis. In order to analyze the mechanisms of the neutrophil and eosinophil migration into the site of anaphylactic reactions in skin, in vitro antigen-evoked release of neutrophil chemotactic factor (NCF) and eosinophil chemotactic factor (ECF) from skin was investigated.

The abdominal skin of guinea pig sensitized with egg albumin was cleaned of subcutaneous tissue and cut into 0.5-mm-thick slices. The slices were incubated with 50 µg/ml egg albumin at 37 °C for 20 min, and NCF and ECF activity in the supernatant were measured with a modification of Boyden technique [1]. using guinea pig neutrophils and eosinophils as indicator cells, respectively. The antigen-evoked NCF and ECF activities were detected in the supernatant. The results of Sephadex G-10 or G-25 gel filtration of the supernatant indicated that the molecular weight of the NCF and ECF were between 300 and 1,300. The release of the NCF and ECF was abolished in the absence of calcium in the reaction mixture. Furthermore, the release of NCF and ECF was suppressed by preincubating the slices with nordihydroguaiaretic acid (50 µM), a 5-lipoxygenase inhibitor, and enhanced with indo-methacin (8.5 µM), a cyclooxygenase inhibitor. The NCF and ECF partially purified by Sep-Pak C18 cartridge had the same retention time as leukotriene B₄ (LTB₄) on reverse-phase HPLC. The significant release of LTB₄ by antigen from sensitized guinea pig skin was ascertained by radioimmunoassay.

These results suggest strongly that the NCF and ECF detected in the present experiment may be LTB₄.

Similar results were obtained in the experiments with human skin. The normal skin obtained from mastectomy was cut into slices, and the slices were passively sensitized with reaginic serum obtained from a mite allergen-sensitive patient. The sensitized skin slices were washed well and incubated in Tyrode solution containing 50µg/ml mite antigen at 37 °C for 20 min. The NCF was released by antigen from sensitized human skin, and the retention time of the NCF was the same as that of LTB₄ on HPLC.

These results suggest that the accumulation of inflammatory cells such as neutrophils and eosinophils might be due to LTB₄ released during cutaneous anaphylaxis and the chemotactic factor, such as LTB₄, might contribute to produce the skin lesion in anaphylactic reaction in concert with other mediators.

Reference

Boyden S: The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. *J Exp Med*, 1962; 115:453–466.