Enhanced skin reactivity to platelet-derived permeability factor (PDPF) and exogenous histamine in an acute phase rabbit model

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Abstract
Rabbits made acute phase by sub-cutaneous trauma with 2% croton oil (in mineral oil) were tested by intradermal (ID) injection with platelet-granule extracts containing platelet-derived permeability factor (PDPF). Compared with controls, skin reactivity to PDPF was enhanced in acute phase animals 3-7 days post-trauma, a period of acute inflammation as reflected by the occurrence in the circulation of C-reactive protein; maximal skin responses were observed 3-4 days post-trauma. Individual skin sites reached maximum intensity 15 min-1 hour post-ID injection of PDPF and were sensitive to chlorpheniramine maleate, suggesting a major role for histamine. Intradermal injection of histamine revealed that acute phase animals yielded an initially more intense skin reaction, and were markedly less capable of recovering from the effects of histamine. These data suggest that in the acute phase, there exists a heightened and prolonged sensitvity to the action of histamine which can be exploited by pro-inflammatory agents such as PDPF.

Introduction
A substantial body of evidence supports a pro-inflammatory role for the platelet. One constituent of platelets, stored in the α-granules and originally described by PACKHAM et al. [1] and NACHMAN et al. [2], is a cationic protein(s) which has the capacity to induce mast cells to release histamine and to produce an anti-histamine sensitive skin reactivity [2, 3]. Our laboratory has a continuing interest in the regulatory mechanisms invoked during the acute phase of inflammatory trauma, and questioned whether the acute phase state might influence the in vivo biology associated with this platelet-derived permeability factor (PDPF). The results of these studies are presented herein.

Materials and methods
Preparation of PDPF (from Nachman et al., 1972)
PDPF was prepared according to the schematic in Fig. 1. Platelets were obtained from the Blood Bank at Rush Medical Center, and isolated as described previously [4].

Rabbits
Rabbits were New Zealand Whites, of mixed sex, weighing 2.5 kg at the start of each experiment. Inflammatory trauma was initiated by sub-cutaneous injection of 2% croton oil (in mineral oil), 3 ml in each flank; non-inflamed controls were not injected. Blood, when required, was obtained by cardiac puncture. PDPF (or a vehicle control, where warranted) was injected intradermally (ID), as was histamine. Skin site reactivity was determined as the square (in mm) of the horizontal and longitudinal diameters. In studies using chlorpheniramine maleate (CM), CM was injected ID 30 minutes prior to the same-site injection of PDPF or histamine [2, 3].

Preparation and quantification of C-reactive protein (CRP)
Rabbit CRP was prepared as previously described [5]. Anti-serum to rabbit CRP was kindly supplied by Dr. Joan Siegel, Department of Immunology/Microbiology, Rush Medical Center, Chicago, Illinois 60612, USA, and was used to prepare plates for radial immunodiffusion to quantitate serum CRP levels. Fused rocket analyses of circulating CRP levels were kindly performed by the clinical service of the Department of Immunology, Institut Pasteur de Lyon, France, using the supplied anti-sera.

Results
Temporal kinetics of the enhanced skin reactivity to PDPF
In the first experiment, inflamed and control rabbits were sequentially injected ID with PDPF on day 2, 7 and 14 post-trauma; the respective skin sites were read over a 2 hour period.
PREPARATION OF PDPF (FROM NACHMAN ET AL., 1972)

**ISOLATED PLATELETS (OR α GRANULES)**

1. **EXTRACT** with H$_2$SO$_4$, **CENTRIFUGE**, **LYOPHILIZE**
2. **DISSOLVE** in DIStilled H$_2$O
3. **PUT OVER** DEAE-CELLULOSE (10mM PHOSPHATE BUFFER, pH 8.0)
4. **COLLECT** Pass-through, Adjust for pH/SALT
5. **SKIN TEST** in RABBITS
6. **POOL** active fractions (FIG. 1), **LYOPHILIZE**, **DISSOLVE** in DIStilled H$_2$O
7. **DIALYZE** vs. DIStilled H$_2$O
8. **CHROMATOGRAPHY** on G-75 (5mM ACETATE BUFFER, pH 4.0)
9. **COLLECT** fractions, Adjust for pH/SALT
10. **SKIN TEST** in RABBITS
11. **POOL** active fractions (FIG. 1)
12. **LYOPHILIZE**, **DISSOLVE** in DIStilled H$_2$O
13. **DIALYZE** vs. DIStilled H$_2$O
14. **BRING** to 0.175m PBS (0.15M NaCl)
15. **PH** 7.4 for Experiments

**Figure 1**
Isolation of PDPF as derived from Nachman et al., 1972.

following the administration of PDPF. As depicted in Fig. 2, there existed a heightened skin reactivity to PDPF in the croton oil-treated animals versus controls on days 2 and 7, but not day 14. To better titrate this effect, we evaluated a group of 64 rabbits, equally distributed between croton-treated and control groups; 4 rabbits from each group were challenged ID with PDPF on days 0–7. Evident in Fig. 3 is that heightened skin reactivity to PDPF commenced between days 2–3, peaked on day 4 and was substantially reduced by day 7; by day 8 there were no differences between the two groups (data not shown). As a measure of the inflammatory response in the rabbits, we determined circulating concentrations of the acute phase reactant, C-reactive protein (CRP). Peak CRP levels in experimental animals, often in excess of 200 μg/ml, occurred on day 2 post-trauma, were generally decreasing by day 4 and had quartered or halved by days 7–8. Thus, onset of the

**Figure 2**
Skin reactivity of rabbits to multiple, sequential ID injection of PDPF. Data are presented as diameter squared ($D^2$ in mm) versus days following trauma. Trauma was initiated by injection of 2% croton oil, as presented in Materials and Methods. PDPF was injected in 100 μl volumes at a concentration suitable to elicit a $D^2$ of 300–400 from non-croton oil-inflamed rabbits. Arrows indicate days of PDPF injection and dotted horizontal bar represents maximum skin bleb immediately following injection. Ranges are given for peak values. A total of 16 rabbits were used in this experiment, equally distributed in the experimental or control groups.