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Reactions in Canine Skin with Human Leukocyte Lysates[1] (39915)

RUSSELL H. TOMAR[2] AND JAMES TERZIAN

Division of Clinical Pathology, Departments of Pathology and Medicine, Upstate Medical Center, Syracuse, New York 13210

Introduction. Human leukocytes transfer delayed hypersensitivity reactions from sensitized donors to immunologically naive recipients. The materials responsible for this activity are present in the dialysate of disrupted white cells. The lack of acceptable assays and animal models has impeded research on this material, which Lawrence called transfer factor (1). In an attempt to develop such an assay, we have been successful in evoking two types of local skin reactions in dogs using fractions of human leukocyte lysates.

Materials and methods. Leukocyte lysates have been prepared from donors who were skin tested to SK-SD and PPD (2). Leukocytes, $1\text{-}2 \times 10^9$, are obtained from 1 unit of blood by sedimentation in 1% dextran. The cells are frozen and thawed 10 times and the cellular debris is removed by centrifugation. The supernatant is ultrafiltered through an XM-50 (Amicon) membrane. This "crude" preparation is further fractionated on a 1.5 × 50-cm P-2 (Biogel) column with a flow rate of 15–20 ml/hr, using 0.001 *M* ammonium carbonate or 0.01 *M* sodium chloride as solvent. Absorbances of the eluates at 260 and 280 nm are determined. The three to five peaks are individually recombined, lyophilized, and refiltered. Peak IV elutes at 1.6 (v/v) glucose, has a high 260/280 ratio, and appears similar to TF fractions described by Zuckerman *et al.* (4), as well as Sandler *et al.* (5). Analyses of this fraction have shown it to transfer skin reactions in men, and to contain hypoxanthine, a variable amount of orcinol-reactive substances (0–9.4 mg/ml of ribose equivalent in material with an optical density of 1.00 at 260 nm), and relatively constant amounts of fluorescamine-reactive materials (2–3 mg/ml of phenylalanine equivalent in material with an optical density of 0.850 at 260 nm) (2, 3, 6, 7). Streptokinase-streptodornase (SK-SD), 100/25 units/ml (Varidase, Lederle), purified protein derivative (PPD), 10 or 5 TU/0.1 ml (Connaught), and coccidiodin (Cutter), respectively, have each served as antigens, and pyrogen-free saline has been used as control. Seven preparations of leukocyte lysates have been tested, five from individual donors and two pooled preparations from three donors. We have tested materials on both mongrels and inbred litter-mate beagles. Dogs were not skin tested prior to injection of the leukocyte lysates.

Our protocol has been to inject 0.1 ml of sterile, filtered endotoxin-negative (limulus lysate, Pyrostat, Worthington) test material intradermally into the well-shaven flank of a dog. Normal saline is also injected into another site as a control. The sites are circled and examined at 4 and 24 hr for induration and erythema. After the reaction has subsided, usually 24–30 hr after the first injection, 0.1-ml samples of antigen are injected into the same circled areas. These sites are examined again at 6, 24, 30, and 48 hr after antigen injection and induration is measured in two diameters. Skin biopsies were taken at several time intervals from a series of both test and control animals. The slides were coded and the histopathology was reviewed without knowledge of the protocol.

Results. Skin reactions occurred after both injections. The first occurred within 4 hr after the injection of leukocyte lysate fractions and generally subsided by 24–30 hr. These were erythematous and indurated. The mean ± SEM diameter of indur-

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[2] Send reprint requests to Russell H. Tomar, M.D., Clinical Pathology, Upstate Medical Center, 750 E. Adams St., Syracuse, New York 13210.

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TABLE I. POSITIVE SKIN REACTIONS IN DOGS WITH COLUMN FRACTIONS OF LEUKOCYTE LYSATES.

P-2 Column fractions[a]	Elution volume, (v/v) glucose	Early response				Antigen-related response			
		No. positive[b]	No. of sites injected	No. of dogs	Percentage positive	No. positive[c]	No. of sites injected	No. of dogs	Percentage positive
I[d]	0.4	11	19	6	58	5	6	5	83
II	0.9	14	39	11	36	9	22	10	41
IIa	1.0								
III	1.4	5	8	3	62	2	6	3	33
IV	1.6	47	53	10	89	10	13	8	77
V	1.8	0	5	3	0	0	5	3	0
Saline	–	1	35	32	3	0[e]	35[f]	32	0

[a] Fractions derived from individuals sensitive to test antigen.
[b] Induration ≥ 5 mm 4 hr after injection of antigen.
[c] Induration ≥ 5 mm 24-48 hr after injection of antigen.
[d] Also void volume (blue dextran).
[e] Saline injected after leukocyte lysate fractions.
[f] Summarizes all fractions (I, II, IIa, III, IV, and V).

ation of all positive reactions using the largest response per test was 7.2 ± 0.8 mm. No such reactions were seen after saline. While fraction IV consistently evoked reactions, other fractions, especially I, were also positive (Table I). There appeared to be no significant difference in the vigor of response caused by fractions I and IV (6.8 ± 1, 7.6 ± 1 mm). Skin biopsies at the height of the early reaction demonstrated a mild subcutaneous polymorphonuclear and mononuclear infiltrate. (Fig. 1A) The second visible reaction peaked at 24–30 hr after injection of antigen. These reactions looked similar to the early ones, i.e., with erythema and induration. The mean ± SEM diameter of induration (7.0 ± 0.6) also was similar to that noted in the early reaction. Histologically, this reaction was characterized by a mild focal perivascular mononuclear infiltrate (Figs. 1B and C) Saline controls were negative (Fig. 1D, Table II).

We tried to ascertain the antigen specificity of the second reaction by using fraction iv preparations from donors with (a) positive delayed skin reactions to SK-SD, but negative to PPD, and (b) positive PPD, but negative coccidiodin reactions. The former preparation gave positive SK-SD, but negative PPD, responses in two dogs, and the second preparation evoked positive PPD, but negative coccidiodin, responses in two dogs. Occasionally, a preparation did not produce a positive reaction to an antigen to which the donor was known to be positive (false negative).

We have tested amounts of endotoxin (5–20 ng/ml) and uric acid (20–50 μg/ml which we might have been unable to readily detect in our preparations. These materials provoked no visible or palpable response in our test animals, although larger concentrations did induce violent local reactions. While we did not attempt to produce systemic transfer, our saline control injection sites were never positive after antigen challenge, suggesting that we did not produce systemic transfer.

Discussion. We have observed two types of skin reactions in association with leukocyte lysate injections in dogs. The early response occurs within 4 hr after injection and subsides by 24–30 hr. This reaction does not require the presence of antigen. Subcutaneous mononuclear and polymorphonuclear leukocytes are seen on biopsy specimen. The second response appears to be antigen specific and peaks 24–30 hr after introduction of antigen. An accumulation of mononuclear cells, particularly around vessels, characterizes such reactions. Gottlieb and his co-workers have also reported dual skin reactions in human subjects with their leukocyte preparations (8, 9). We are not certain of the relationship of the early to late response, but we have noted that an early reaction is not a prerequisite for the later, antigen-related response. Conversely, the appearance of an early reaction does not ensure a later one after antigen challenge. Thus, it seems likely that the earlier reaction is due to an unknown "contami-

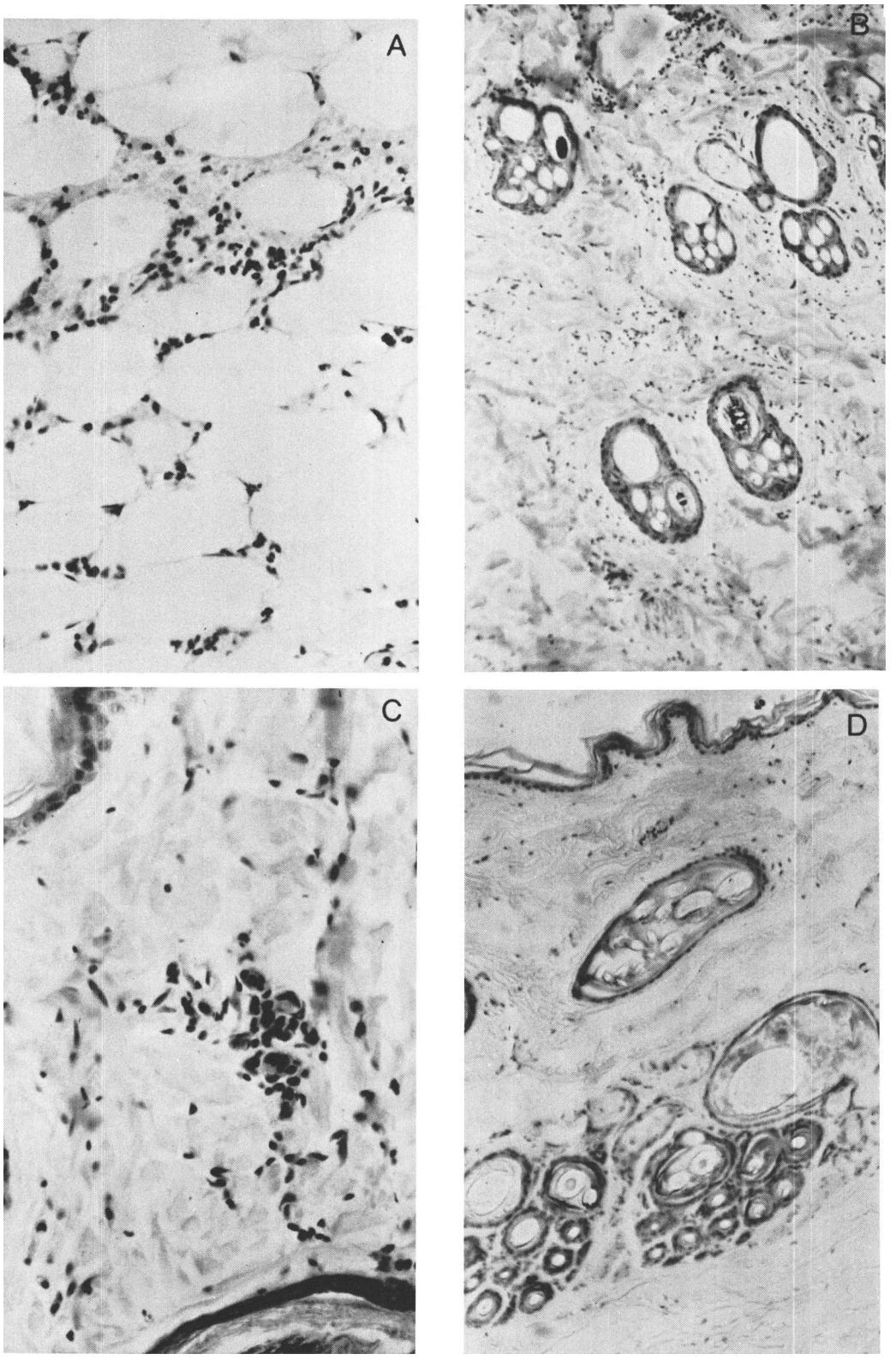

FIG. 1. (A) A moderate subcutaneous polymorphonuclear infiltration is demonstrated 4 hr after injection of leukocyte lysate Fraction IV. ×1350. (B) (C) Low- and high-power views of the mild focal perivascular mononuclear infiltrate, which occurs in the dermis 24–30 hr after injection of antigen, are shown. ×300 and ×1350, respectively. (D) Saline control reveals no inflammatory reaction. ×300.

TABLE II. SUMMARY OF HISTOPATHOLOGICAL REACTIONS IN SKIN BIOPSIES.

(A) Intradermal (id)	(B) Intradermal	(C) Biopsy: Hours after (A)	Findings
(1) Saline	–	4	No significant abnormality
(2a) Fraction IV[a]	–	4	Mild subcutaneous polymorphonuclear and mononuclear infiltrate
(2b) Fraction IV[a]	–	24	Moderate diffuse subcutaneous polymorphonuclear infiltrate
(2c) Fraction IV[a]	Saline 24 hr later	48	Mild scattered subcutaneous mononuclear and polymorphonuclear infiltrate
(3a) Fraction IV[a]	SK-SD 24 hr later	48	Mild focal perivascular mononuclear infiltrate in subcutaneous region
(3b) Fraction IV[a]	SK-SD 24 hr later	72	Mild scattered mononuclear and polymorphonuclear subcutaneous infiltrate
(3c) Fraction IV[a]	PPD intermediate 24 hr later	48	No significant abnormality
(4) Saline	SK-SD 24 hr later	48	No significant abnormality

[a] Leukocyte lysate fraction IV from a SK-SD positive, PPD-negative donor.

nant" of our preparations. We find skin test activity in two separate peaks from the P-2 column, with some "smearing" into the nearest fractions. The relationship of these fractions is completely unknown. It is tempting to postulate that a smaller molecule in peak IV is the active piece of a larger one in peak I. While we believe that the canine local transfer procedure may provide an assay for transfer factor, we must caution that the same fractions active in dogs have not yet been tested in man.

Summary. Two types of local reactions in canine skin were observed after intradermal injection of chromatographically prepared fractions of human perpheral blood leukocyte lysates. The first reaction peaked within 4 hr after injection, appeared unrelated to antigen, and was characterized by a mild diffuse mononuclear and polymorphonuclear infiltrate in the subcutaneous tissue. The second response peaked 30 hr after the injection of antigen onto the site previously prepared with leukocyte lysate. This response appeared to be antigen specific, and was characterized by an early perivascular mononuclear infiltrate followed by a diffuse mononuclear and polymorphonuclear accumulation in the subcutaneous region. Peaks I and IV most consistently gave positive results.

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