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The role of complement, platelet-activating factor and leukotriene B_4 in a reversed passive Arthus reaction

[1]A.G. Rossi, K.E. Norman, *D. Donigi-Gale, *T.S. Shoupe, R. Edwards & T.J. Williams,

Department of Applied Pharmacology, National Heart & Lung Institute, Dovehouse Street, London SW3 6LY and *Department of Molecular Discovery, Purdue Frederick Company, Norwalk, Connecticut, U.S.A.

1 The mechanisms underlying oedema formation induced in a reversed passive Arthus (RPA) reaction and, for comparison, in response to zymosan in rabbit skin were investigated.

2 Oedema formation at skin sites was quantified by the accumulation of intravenously-injected ^{125}I-labelled human serum albumin.

3 Recombinant soluble complement receptor type 1 (sCR1), administered locally in rabbit skin, suppressed oedema formation induced in the RPA reaction and by zymosan.

4 The platelet-activating factor (PAF) antagonists, WEB 2086 and PF10040 administered locally, inhibited oedema formation induced in the RPA reaction and by PAF but not by zymosan.

5 A locally administered leukotriene B_4 (LTB_4) antagonist, LY-255283, inhibited oedema formation induced by LTB_4 but did not inhibit oedema responses to PAF, zymosan or the RPA reaction.

6 The results demonstrate a role for complement in oedema formation in both the RPA reaction and in response to zymosan. An important contribution by PAF is indicated in the RPA reaction but not in response to zymosan whereas no evidence was obtained to suggest a role for LTB_4 in either inflammatory response.

Keywords: Reversed passive Arthus reaction; complement; soluble complement receptor type 1 (sCR1); platelet-activating factor (PAF); leukotriene B_4 (LTB_4); oedema formation

Introduction

Maurice Arthus at the turn of this century first described the acute inflammatory and haemorrhagic reaction produced in the skin of rabbits when a local injection of horse serum was administered to previously sensitized rabbits (Arthus, 1903). Although much progress has been made in the elucidation of the mechanisms involved in this complex phenomenon, the mechanisms remain only partially understood (Humphrey, 1955; Cochrane & Janoff, 1974; Williams *et al.*, 1986; Hellewell, 1990). Experimentally it is convenient to investigate the reversed passive Arthus (RPA) reaction. This inflammatory reaction differs from the direct Arthus reaction (described above) in that it is elicited by an intradermal (i.d.) injection of antibody and an intravenous (i.v.) injection of the corresponding antigen. These Arthus reactions, classified as Type III hypersensitivity reactions, are models of vascular injury which are initiated by the deposition of antigen-antibody complexes within the wall of skin microvessels. The ensuing inflammatory reaction is characterized by oedema formation, neutrophil accumulation, platelet accumulation and haemorrhage. In severe reactions the inflammatory response culminates in tissue necrosis.

Early observations showed the depletion of circulating neutrophils with nitrogen mustard or anti-neutrophil antiserum severely depressed oedema in the Arthus reaction suggesting that these cells play a crucial role in this inflammatory reaction (Stetson & Good, 1951; Humphrey, 1955). In addition, systemic depletion of the complement system with cobra venom factor also suppresses the Arthus reaction (Cochrane *et al.*, 1970; Cochrane & Janoff, 1974). A key mediator in the Arthus reaction may be the complement protein fragment C5a which is a potent chemoattractant for neutrophils. C5a was also shown to be potent in inducing oedema formation in the skin (Williams & Jose, 1981). Further, the oedema response although evident very early after injection (5–6 min) was totally inhibited following depletion of circulating neutrophils (Wedmore & Williams, 1981b). In RPA reactions induced in the rabbit peritoneal cavity, C5a has been detected in inflammatory exudate by use of radioimmunoassay (Jose *et al.*, 1983).

We have investigated the role of complement in the RPA reaction and in zymosan-induced oedema formation using the recombinant soluble human complement receptor type I (sCR1) (Weisman *et al.*, 1990; Yeh *et al.*, 1991). The single chain membrane bound glycoprotein, CR1 (C3b/C4b receptor; CD35), exerts a number of inflammatory regulatory functions in the body (Fearon & Wong, 1985; Ross & Medof, 1985; Molines & Lachmann, 1988). In addition, CR1 inactivates C3 and C5 convertases, thereby controlling the activation of the classical and the alternative pathways of the complement cascade (Iida & Nussenzweig, 1981; Weisman *et al.*, 1990; Yeh *et al.*, 1991). Thus, an active soluble form of CR1 (sCR1) may have therapeutic benefits in many inflammatory disease states where activation of the complement cascade is prominent.

Several membrane-derived lipids have been implicated as important mediators of Arthus reactions (Hellewell & Williams, 1986; Williams *et al.*, 1986). The arachidonic acid metabolite, prostaglandin E_2 (PGE_2) injected i.d. alone into rabbit skin results in little plasma leakage; however, when co-injected with agents that increase microvascular permeability the eicosanoid, by virtue of its vasodilator properties, acts synergistically to augment oedema formation (Williams & Peck, 1977; Wedmore & Williams 1981b). It was subsequently demonstrated that local treatment with the cyclooxygenase inhibitor indomethacin, suppresses the Arthus reaction, an inhibition which can be reversed by local administration of PGE_2 (Williams *et al.*, 1986). The precise contribution made by two other important proinflammatory agents; the ether lipid platelet-activating factor (PAF) and the 5-lipoxygenase product leukotriene B_4 (LTB_4), in the

[1] Author for correspondence.

Arthus reaction, still remains to be established. In this study we therefore investigated the involvement of complement, PAF and LTB_4 in the RPA reaction and, for comparison, the response to zymosan in the rabbit skin. The agents used were sCR1 (Weisman *et al.*, 1990) the PAF antagonists, WEB 2086 (Casals-Stenzel *et al.*, 1987) and PF10040 (Rossi *et al.*, 1992) and the LTB_4 antagonist, LY-255283 (Jackson *et al.*, 1988; Snyder & Fleisch, 1989).

Methods

Animals

Male New Zealand White rabbits (2–3.5 kg) were purchased from Froxfield Farm, Hampshire.

Generation of antiserum for Arthus reactions

Arthus antiserum, anti-bovine-γ-globulin (anti-BGG) was raised in rabbits as previously described (Hellewell & Williams, 1986). Briefly, subcutaneous injections (4 × 0.25 ml) of BGG (2 mg ml^{-1} in saline) emulsified with an equal volume of Freund's complete adjuvant were administered. This was followed 14 days later by booster subcutaneous injections (4 × 0.25 ml) of the same concentration of BGG in Freund's incomplete adjuvant. At day 28 a subcutaneous injection of alum-precipitated BGG (300 μg/rabbit) was given. Blood was collected by carotid cannulation at day 38, the serum from five rabbits was pooled, heat-inactivated at 56°C for 30 min and stored in aliquots at − 20°C. Heat-inactivated normal rabbit serum was used as the control.

Preparation of zymosan activated plasma (ZAP)

ZAP (a source of C5a des Arg) was prepared by incubating heparinised (10 u ml^{-1}) rabbit plasma with zymosan (5 mg ml^{-1}) for 30 min at 37°C. Zymosan was removed by centrifugation (2 × 10 min, 2500 *g*) and ZAP stored in 1 ml aliquots at − 20°C. The C5a des Arg content of ZAP was approximately 5×10^{-7} M as measured by radioimmunoassay (Collins *et al.*, 1991).

Measurement of local oedema formation in rabbit skin

Rabbits were anaesthetized with i.v. sodium pentobarbitone and the dorsal skin clipped and marked out with 16 treatment skin sites in 6 replicates per animal according to a balanced site plan. Plasma leakage was measured by i.v. injection of ^{125}I-labelled human serum albumin (5 μCi kg^{-1}) together with the visual marker Evans Blue dye (10 mg kg^{-1}) in saline as previously described (Wedmore & Williams, 1981b). After 10 min the agents under investigation, freshly prepared in sterile isotonic saline (unless otherwise stated), were injected i.d. in 0.1 ml volumes. Where indicated, agonists were co-injected with PGE_2 (3×10^{-10} mol/site) to facilitate the measurement of microvascular plasma protein leakage (Wedmore & Williams, 1981b). Cardiac blood samples were collected 4 h after the i.d. injections into heparinised test tubes for the preparation of plasma. The animals were then killed by an overdose of pentobarbitone the dorsal skin removed and the skin sites excised with a 17 mm diameter punch. Radioactivity in the skin sites and in 1 ml plasma samples were counted in an automatic gamma counter. Results are expressed as μl plasma per site by dividing skin sample ^{125}I-counts by ^{125}I-counts in 1 μl of plasma.

For the reversed passive Arthus reaction, 10 min after the i.v. injection of radiolabelled albumin, 0.1 ml volumes of anti-BGG antiserum (undiluted, diluted 1/2, 1/4 and 1/8 with saline) were injected i.d., followed 5 min later by an i.v. injection of the antigen (BGG; 5 mg kg^{-1}). Oedema formation was assessed after 4 h as described above.

Materials

Sagatal (pentobarbitone sodium, 60 mg ml^{-1}) was purchased from May and Baker, Dagenham, Essex. Bovine serum albumin, BGG and zymosan were from Sigma Chemical Co., Poole, Dorset. [^{125}I]-labelled human serum albumin (20 mg albumin per ml of sterile isotonic saline, 50 μCi ml^{-1}) was from Amersham International plc, Amersham, Buckinghamshire. Freund's complete and incomplete adjuvant were from Difco Laboratories, West Molesey, Surrey, Evans blue was from British Drug House, Poole, Dorset. Viaflex (sterile, pyrogen-free isotonic saline solution) was from Baxter Healthcare Ltd, Thetford, Norfolk. Sterile pyrogen-free water was from Phoenix Pharmaceuticals Ltd., Gloucester. Human sCR1 was genetically engineered by site directed mutagenesis and secreted from transfected Chinese hamster ovary cells (Yeh *et al.*, 1991) and was a kind gift from SmithKline & Beecham, Epsom, Surrey. PAF (1-0-hexadecyl-2-acetyl-*sn*-glycero-3-phosphorylcholine) was dissolved in 1% BSA in saline and was purchased from Bachem, Saffron, Walden, Essex. LTB_4 [5(**S**),12(**R**)-dihydroxy-6, 14-*cis*-8,10-trans-eicosatetraenoic acid] was purchased from Cascade Biochem Ltd., Reading, Berkshire, and was a gift from Dr R.M. McMillan, ICI Pharmaceuticals, Alderley Park. PF10040 (1-(3,4-dimethoxyphenylethyl)-6-methyl-3, 4-dihydroisoquinoline hydrochloride) was a gift from Purdue Frederick, Norwark, Connecticut, U.S.A. LY-255283 [1-(5-ethyl-2-hydroxy-4- (6-methyl-6-(1H-tetrazol-5-yl)-heptyloxy) phenyl) ethanone], initially dissolved in 0.01 M NaOH then sequentially diluted in saline, was a gift from Lilly Research Laboratories, Indianapolis, Indiana, U.S.A. WEB 2086 (3-[4-(2-chlorophenyl) -9-methyl-6H-thieno [3,2-f] [1,2,4]-triazolo-[4,3-a] [1,4]-diazepine-2-yl]-1- (4-morpholinyl) -1-propanone) was a gift from Boehringer Ingelheim, Bracknell, Berkshire.

Statistical analysis

The data are presented as the mean ± s.e.mean and have been analyzed by two way analysis of variance. Significant differences ($*P<0.05$, $**P<0.01$) between groups were determined by the Neuman-Kuels procedure.

Results

Effect of sCR1 on plasma leakage

Intradermal injections of zymosan (3–300 μg/site) elicited a dose-dependent oedema formation as measured by the leakage of ^{125}I-human serum albumin at skin sites (Figure 1). In the presence of sCR1 (3 μg/site) these responses were significantly attenuated ($n = 3$ rabbits). Undiluted ZAP, as a source of C5a, mixed with PGE_2 (3×10^{-10} mol/site) induced a response of 126.8 ± 15.1 μl plasma leakage which was unaffected by co-injection of sCR1 (123.1 ± 14.9μl). Injection of sCR1 and PGE_2 alone induced little oedema formation.

In order to determine the role of complement in the RPA reaction we examined the effect of sCR1 on oedema produced by different titres of Arthus antiserum (Figure 2). Arthus antiserum titres (12.5–100%) induced a dose-dependent oedema formation, which in the presence of sCR1 was significantly reduced ($n = 7$ rabbits). Responses to non-immune serum and sCR1 alone were minimal i.e., 2.7 and 1.9 μl above the saline control respectively. The response to zymosan (300 μg/site) in these experiments was 55.6 ± 9.7 μl and with co-injection of sCR1 this was reduced to 38.7 ± 9.7 μl ($P<0.01$; $n = 8$). Note that sCR1, at a concentration of 10 μg/site, also did not affect the response to ZAP plus PGE_2 (data not shown).

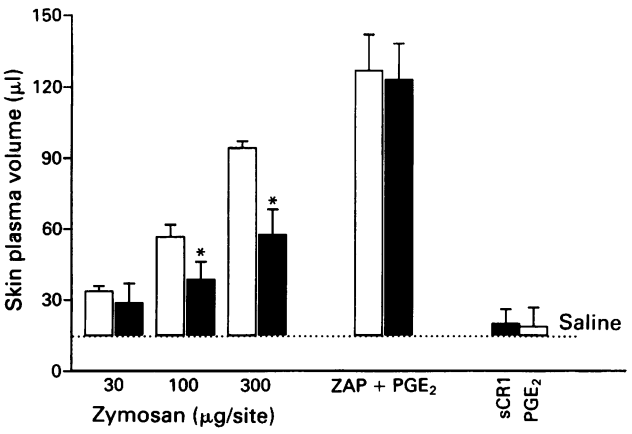

Figure 1 Effect of recombinant soluble complement receptor type I (sCR1) on oedema formation induced by zymosan and zymosan activated plasma (ZAP) plus prostaglandin E_2 (PGE_2) in rabbit skin. Intradermal (i.d.) injections of 30, 100 and 300 μg/site zymosan and undiluted ZAP plus PGE_2 were given alone (open columns) or in the presence of 3 μg/site sCR1 (solid columns). The dotted line shows the control value obtained after i.d. injection of saline. The results are expressed as mean (± s.e.mean, vertical bars) μl plasma volume values from $n = 3$ animals. The significant inhibitory effect of sCR1 is shown as $*P < 0.05$.

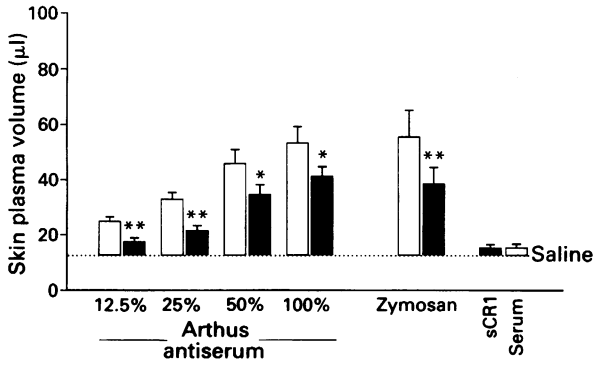

Figure 2 Effect of recombinant soluble complement receptor type I (sCR1) on oedema formation induced by zymosan and produced in the reversed passive Arthus (RPA) reaction in rabbit skin. Intradermal (i.d.) injections of 300 μg/site zymosan and RPA antiserum titres of 12.5%, 25%, 50% and 100% administered alone (open columns) or in the presence of 10 μg/site sCR1 (solid columns). The dotted line shows the control value obtained after i.d. injection of saline. The results are expressed as mean (± s.e.mean, vertical bars) μl plasma volume values from $n = 7$–9 animals. The significant inhibitory effect of sCR1 is shown as $*P < 0.05$ or $**P < 0.01$.

Effect of the PAF antagonists, PF10040 and WEB 2086, on plasma leakage

The next series of experiments was designed to investigate the involvement of PAF in the above inflammatory responses. We therefore examined the effects of the compounds PF-10040 and WEB 2086 on oedema produced in the RPA reaction and, for comparison, oedema induced by PAF plus PGE_2 and zymosan (Figure 3). Plasma leakage induced by PAF (10^{-9} mol/site) plus PGE_2 (3×10^{-10} mol/site) was 45.1 ± 7.6 μl; this response was reduced to 22.5 ± 1.4 μl ($P < 0.01$; $n = 6$ rabbits) by PF10040 (10^{-7} mol/site) and to 17.5 ± 1.9 μl ($P < 0.01$; $n = 6$ rabbits) by WEB 2086 (10^{-7} mol/site). The compounds alone or in the presence of PGE_2 induced little or no oedema formation. In these experiments it was observed that the RPA reaction induced a response of 33.2 ± 6.6 μl; this response was reduced to 22.5 ± 5.5 μl ($P < 0.01$; $n = 5$ rabbits) by PF10040 (10^{-7} mol/site) and to 22.4 ± 3.4 μl ($P < 0.01$; $n = 5$ rabbits) by WEB 2086 (10^{-7} mol/site). For comparison, oedema formation induced by zymosan (300 μg/site) was unaffected ($n = 6$ rabbits) by co-injection of either PAF antagonist.

Effect of the LTB_4 antagonist, LY-255283, on plasma leakage

Figure 4 clearly shows that LY-255283 at 10^{-7} mol/site suppressed the response to LTB_4 (5×10^{-10} mol/site) from 29.9 ± 5.1 μl to 17.6 ± 2.4 μl ($P < 0.01$; $n = 6$ rabbits) and the response to LTB_4 (5×10^{-10} mol/site) plus PGE_2 (3×10^{-10} mol/site) from 54.9 ± 13.7 μl to 19.5 ± 3.1 μl ($P < 0.01$; $n = 6$ rabbits). Oedema formation induced by zymosan (300 μg/

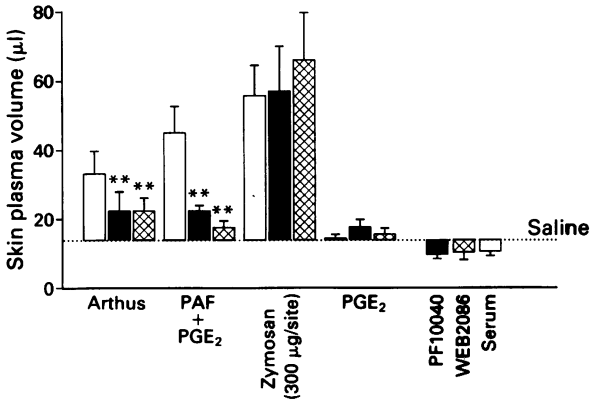

Figure 3 Effect of the platelet-activating factor (PAF) antagonists, PF10040 and WEB 2086 on oedema formation produced in the reversed passive Arthus (RPA) reaction and induced by PAF plus prostaglandin E_2 (PGE_2) and zymosan. RPA antiserum (undiluted), PAF (10^{-9} mol/site) plus PGE_2 (3×10^{-10} mol/site) and zymosan (300 μg/site) injected alone (open columns), with 10^{-7} mol/site PF-10040 (solid columns) or with 10^{-7} mol/site WEB 2086 (hatched columns). The dotted line shows the control value obtained after i.d. injection of saline. The results are expressed as mean (± s.e.mean, vertical bars) μl plasma volume values from $n = 5$–6 animals. The significant inhibitory effect of the PAF antagonists is shown as $**P < 0.01$.

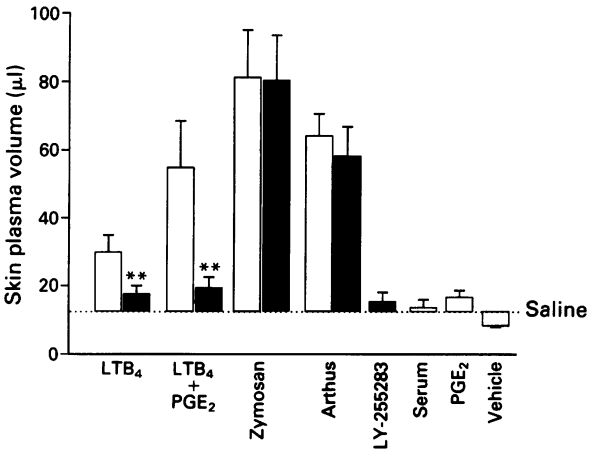

Figure 4 The effect of the leukotriene B_4 (LTB_4) antagonist, LY-255283 on oedema formation produced in the reversed passive Arthus (RPA) reaction and induced by LTB_4, LTB_4 plus prostaglandin E_2 (PGE_2) and zymosan. RPA antiserum (undiluted), LTB_4 (5×10^{-10} mol/site), LTB_4 (5×10^{-10} mol/site) plus PGE_2 (3×10^{-10} mol/site) and zymosan (300 μg/site) were i.d. injected alone (open columns) or with 10^{-7} mol/site LY-255283 (solid columns). The dotted line shows the control value obtained after i.d. injection of saline. The results are expressed as mean (± s.e.mean, vertical bars) μl plasma volume values from $n = 6$ animals. The significant inhibitory effect of LY-255283 is shown as $**P < 0.01$.

site) and the response produced by the RPA reaction were unaffected by co-injection with LY-255283. In a further series of experiments ($n = 7$ rabbits) where LTB_4 plus PGE_2 was similarly inhibited and the Arthus reaction was not affected by the antagonist, the response induced by PAF plus PGE_2 was 57.0 ± 8.1 µl and in the presence of LY-255283, the response was not significantly inhibited and was 53.8 ± 6.4 µl.

Discussion

In this study we investigated the inflammatory mechanisms underlying the Arthus reaction. Specifically, we showed that a truncated and soluble form of CR1 (sCR1) given locally into rabbit skin not only inhibited oedema formation in the RPA reaction, but also suppressed oedema induced by zymosan (Figures 1 and 2). Thus, sCR1 inhibited both the classical pathway of the complement cascade which is activated by the deposition of immune-complexes in the RPA reaction and also inhibited the alternative pathway which is typically activated by yeast cell wall polysaccharides such as zymosan. Previous studies showed that systemic treatment of animals with cobra venom factor or antibodies to complement components suppresses Arthus reactions (Ward & Cochrane, 1965; Cochrane *et al.*, 1970; Cochrane & Janoff, 1974). The present study shows that intradermally-injected complement inhibitor can suppress both the Arthus reaction and the response to zymosan. This emphasizes the importance of local activation of complement in the interstitium in these oedema responses (Williams & Jose, 1981).

Our results are in agreement with a recent paper by Yeh *et al.* (1991). They demonstrated that sCR1 inhibits both the classical and alternative pathways of the complement cascade using an *in vitro* sheep erythrocyte haemolytic assay. Furthermore, using a rat RPA reaction model, they showed that i.d. administration of sCR1 dose-dependently suppresses the inflammatory response as judged by both gross and microscopic examination. These authors also demonstrated by immunological localization of C3 and C5b-9 neoantigen deposition that the immunofluorescence in the RPA reaction in the presence of sCR1 is markedly lower than that produced in the RPA reaction alone. ZAP, as a source of C5a, was included as a control in our experiments described here. Oedema induced by ZAP was unaffected by sCR1 showing that the compound did not interfere with the action of C5a, once formed. These results indicate that complement activation is important in the Arthus reaction and that sCR1 may be a useful inhibitor of the inflammatory response, not only in Type III hypersensitivity reactions but also in other pathological conditions where complement activation is involved. Indeed, Weisman *et al.* (1990) showed that sCR1 had anti-inflammatory activity when administered i.v. in a rat *in vivo* model of reperfusion injury of ischaemic myocardium.

We have previously described experiments indicating that vasodilator prostaglandins are produced in the Arthus reaction and that these mediators have a potentiating role in oedema formation (Williams *et al.*, 1986). Here we have investigated the role of other membrane-derived lipids, namely PAF and LTB_4. Our results show that the PAF antagonists, WEB 2086 and PF10040, specifically inhibit oedema formation produced in the RPA reaction and induced by PAF but not that induced by zymosan (Figure 3). This suggests that PAF plays an important role in the Arthus reaction but not in zymosan-induced plasma leakage. Our results are in accord with a report by Hellewell & Williams (1986) who showed that the PAF antagonist, L-652371, suppresses oedema formation in the RPA reaction. Further evidence supporting a role for PAF in the RPA reaction has been presented by other investigators using different PAF antagonists administered by various routes in a number of animal models (Deacon *et al.*, 1986; Issekutz & Szejda, 1986; Camussi *et al.*, 1987; Warren *et al.*, 1989; Hellewell, 1990).

The role of 5-lipoxygenase products and in particular LTB_4 in the Arthus reaction remains unclear. It has been shown that several 5-lipoxygenase inhibitors given intrapleurally inhibit a reversed passive Arthus pleurisy model in the rat (Berkenkopf & Weichman, 1991) and the 5-lipoxygenase inhibitor, A-63162, suppresses inflammation induced in the RPA reaction in the mouse (Zhang *et al.*, 1991). These observations suggest a role of 5-lipoxygenase products in the Arthus reaction but they do not shed light on the contribution of specific products of 5-lipoxygenase activity (e.g. LTB_4). In the study described here we have shown that local administration of the LTB_4 antagonist, LY-255283, inhibited oedema formation induced by LTB_4 alone and LTB_4 plus PGE_2, but did not affect leakage produced in the RPA reaction or induced by zymosan or PAF (Figure 4). The lack of effect is not due to clearance of LY-255283 from the skin site as LTB_4-induced oedema formation is inhibited when it is administered locally into a site injected 4 h previously with LY-255283 (Von Uexkull *et al.*, unpublished observations). The reported inhibition of the Arthus reaction by various 5-lipoxygenase inhibitors may be due to other bioactive 5-lipoxygenase products such as LTC_4, LTD_4 and LTE_4 or simply non specific effects of the inhibitors. Alternatively, it is possible that LTB_4 is indeed involved in the RPA reaction but that its site of action is not accessible to the antagonist in our model. It is also feasible that the amount of LTB_4 produced in the RPA reaction is so small that its effects are masked by the effects of C5a. Our results with the LTB_4 antagonist, however, suggest that LTB_4 does not play a major role in the induction of oedema in the RPA reaction or in response to zymosan.

A schematic representation of the events that may occur in the RPA reaction is illustrated in Figure 5. Circulating antigen diffuses out of the lumen of the blood vessel and across the microvascular endothelial cells where it meets the antibody, forming immune complexes in the microvessel wall. These complexes activate the classical pathway of the complement cascade resulting in the formation of the chemoattractant C5a. C5a induces neutrophils to adhere to the endothelial cells followed by migration via endothelial junctions. The interaction between neutrophils and endothelial cells triggers increased microvascular permeability by an unknown mechanism (Wedmore & Williams, 1981b). This interaction can explain the effect of the depletion of circulating neutrophils on oedema formation in the Arthus reaction (Stetson & Good, 1951; Humphrey, 1955). The leakage

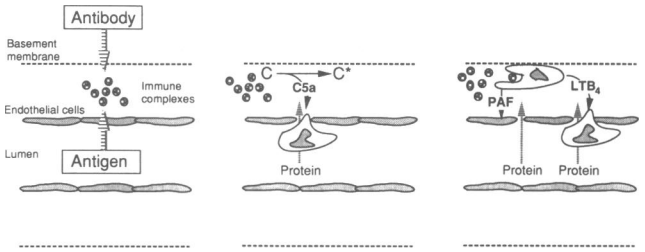

Figure 5 Diagrammatic representation of the possible events occurring in the reversed passive Arthus reaction. Circulating antigen diffuses from the lumen of the micro-blood vessels across the endothelial cell layer where it forms immune complexes with the antibody in the vessel wall. The complexes induce the activation of the classical pathway of the complement cascade leading to the generation of C5a. C5a attracts neutrophils which then adhere to and marginate across the endothelial cell layer resulting in oedema formation. The neutrophils phagocytose the immune complexes and generate PAF. The newly formed PAF then acts directly on the endothelial cells to cause further plasma leakage. The activated neutrophils can also synthesize LTB_4 which may attract other neutrophils to the site of inflammation to produce more oedema formation.

induced provides further antigen to the interstitium thus facilitating further immune complex deposition. Neutrophils that have migrated through the endothelium soon encounter immune complexes under and around the basement membrane and phagocytosis begins. This stimulates the release of PAF from neutrophils which acts directly on endothelial cells to cause further leakage (Wedmore & Williams, 1981a; Braquet *et al.*, 1987). Activated neutrophils may also release LTB_4 which then could attract more neutrophils, although this did not appear to be a major component in this model. Injection of zymosan will also generate C5a, in this case by the alternative pathway of the complement cascade, resulting in plasma leakage. Our results show that sCR1 reduced the plasma leakage produced in both the RPA reaction and in response to zymosan presumably by inhibiting the formation of C5a. In contrast, the PAF antagonists inhibited the oedema formation in the Arthus reaction but did not suppress the response induced by zymosan, suggesting a role for PAF in the former but not in the latter response. An explanation for this observation may be that in the Arthus reaction, phagocytosis takes place when neutrophils are in close contact with endothelial cells. By comparison, when zymosan is injected intradermally subsequent phagocytosis takes place at sites remote from the endothelium. PAF is unstable in tissue fluid and levels in the region of the endothelium may be very low under these circumstances. This argument does not hold for C5a which will also be generated either close to, or remote from the endothelium in the two models. In this case, although there may be some metabolism in tissue fluid (by the action of carboxypeptidase N) the metabolic product C5a des Arg is also highly active as a permeability increasing mediator *in vivo* and is stable in tissue fluid (Jose *et al.*, 1981).

These observations shed some light on the mediators involved in the Arthus reaction. The results illustrate how mediators may be involved in sequence in inflammatory reactions, suggesting that therapeutic agents may be more effective if aimed at the early events in the response. Further, in terms of inflammatory mechanisms, consideration of the exact site of liberation and the stability of an individual mediator is important in determining its relative role in a particular inflammatory reaction.

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