Immunology 1985 **55** 149

Kinetics of tachyphylaxis to mediators of acute inflammation

I. G. COLDITZ *CSIRO, Division of Animal Health, Armidale, NSW, Australia*

Accepted for publication 7 December 1984

Summary. The kinetics of vascular leakage and tachyphylaxis induced by histamine and bradykinin were examined in rabbit skin and were compared with the kinetics of tachyphylaxis of neutrophil accumulation in lesions induced with the chemotaxin formylmethionyl-leucyl-phenylalanine (FMLP). Maximal leakage of ^{125}I-human serum albumin occurred during the first 5 min after injection of bradykinin and from 10 to 20 min after injection of histamine. Tachyphylaxis developed within 30 min of injection of bradykinin and 1 hr after injection of histamine. For both agents, there was a linear regression of sensitivity with time from 8 hr to 4 days, with normal sensitivity estimated to return at 4·6 days for bradykinin and 4·7 days for histamine. Marked cross-desensitization occurred between the two agents, and lesions initiated with the chemotaxin FMLP were desensitized to restimulation with a mixture of histamine and bradykinin. Initiation of lesions with histamine and bradykinin did not diminish the accumulation of neutrophils when lesions were restimulated with FMLP. The kinetics of tachyphylaxis of neutrophil accumulation in lesions stimulated with FMLP exhibited a linear regression of sensitivity on time between 4 days and 10 days, with estimated resensitization at 11·9 days. Histamine and bradykinin induce enhanced vascular permeability when endothelial cells in post-capillary venules contract following stimulation of their membrane receptors for these agents. We have recently proposed that the migration of neutrophils into acute inflammatory lesions is regulated by a mechanism coupled to chemotaxin receptors which may be similarly located on endothelial cells within the lesions. The present experiments indicate that expression of receptors for vascular permeability agents and the chemotaxin FMLP are independent events with distinct kinetics.

Correspondence: Dr I. G. Colditz, CSIRO, Division of Animal Health, Private Mail Bag, Armidale, NSW 2350, Australia.

INTRODUCTION

Experimental acute inflammation is characterized by leakage of plasma constituents and infiltration of neutrophils into the affected tissues. Increased vascular permeability is usually biphasic; there is an early period of leakage lasting up to 30 min (Wilhelm, 1973) which is mediated by permeability agents such as peptides, monoamines and anaphylatoxins. This is followed by a second prolonged period of leakage which is associated with cellular emigration from the inflamed vascular bed (Moses, Geschickter & Ebert, 1968). In recent years, a large number of chemotaxins have been identified which bind to specific receptors on the nuetrophil cytoplasmic membrane and induce oriented locomotion of these cells along a concentration gradient of the mediator *in vitro*. Intradermal injection of chemotaxins induces a characteristically transient influx of neutrophils, with few neutrophils entering lesions more than 4 hr old (Colditz & Movat, 1984a, c), but it is not yet clear how chemotaxins recruit neutrophils which leave inflamed vessels predominantly at post-capillary venules. Recent observations have shown that lesions in the skin of rabbits show tachyphylaxis to the chemotaxin used to initiate the lesion (Colditz & Movat, 1984b). When restimulated several times with the same chemotaxin, a final injection failed to recruit any additional neutrophils into a lesion. In contrast, restimulation of lesions with

a different chemotaxin evoked a normal cellular influx. These findings led to the suggestion that migration of neutrophils into an acute inflammatory lesion is regulated by a mechanism coupled to chemotaxin receptors within the lesion. A likely location for these receptors is the endothelial cells of post-capillary venules (Colditz & Movat, 1984b).

The vascular permeability agents histamine (Heltianu, Simionescu & Simionescu, 1982; Simionescu *et al.*, 1982) and bradykinin (Regoli, 1983) bind to specific receptors on vascular endothelium and induce endothelial cell contraction (Majno, Shea & Leventhal, 1969) and leakage of plasma. With this in mind, the present study examines the response of rabbit skin to repeated stimulation with histamine and bradykinin, and compares it with tachyphylaxis of neutrophil accumulation in lesions stimulated with the chemotaxin formylmethionyl-leucyl-phenylalanine (FMLP).

MATERIALS AND METHODS

Reagents

Histamine, lysine-bradykinin and endotoxin (*E. coli* serotype 055:B5) (Sigma, St Louis, MO) were dissolved in pyrogen-free normal saline and stored in aliquots at −18°. FMLP (Sigma) was dissolved in dimethylsulphoxide at 10^{-2} M and diluted to 10^{-3} M in normal saline as a stock solution. Working concentrations of reagents were prepared by dilution in normal saline.

Quantitation of vascular leakage and neutrophil accumulation in inflammatory lesions

Lesions were induced in the dorsal skin of adult New Zealand white rabbits by injecting 0·2 ml of inflammatory agent intradermally. Five skin sites were used for each treatment. Replicate experiments were conducted in 2–4 rabbits. Plasma leakage was determined by intravenous injection of 25 μCi ^{125}I-labelled human serum albumin (HSA) (Amersham International, Amersham, Bucks). Rabbits were killed with an overdose of pentobarbital at the appropriate time after plasma labelling, skins were removed and blood was manually expressed from the pelt. Neutrophil infiltration of inflammatory lesions was determined as described previously (Colditz & Movat, 1984c). Briefly, autologous or donated peripheral blood neutrophils were purified from leucocyte-rich plasma on a cushion of Percol (Pharmacia, Uppsala, Sweden) to a purity greater than 90% and were labelled at 37° for 30 min with 50 μCi Na_2 [^{51}Cr]O_4 (Amersham) per 5×10^7 cells. Lesions on neutrophil recipients were restimulated 2 hr before euthanasia, and at least 5×10^7 labelled neutrophils were injected intravenously 1 hr before killing. Lesions were excised with a 22-mm diameter hollow punch and radioactivity quantified in a Packard γ spectrometer with window settings 10–90 KeV (^{125}I) and 250–400 KeV (^{51}Cr). ^{125}I and ^{51}Cr counts in unstimulated skin were subtracted from measurements in experimental lesions. In some experiments, leakage of ^{125}I-HSA into lesions was expressed in terms of the specific activity of plasma at killing, yielding an estimate of μl plasma present in lesions. Saline alone caused no significant vascular leakage or neutrophil accumulation.

RESULTS

Dose responses and kinetics of vascular permeability

The leakage of plasma into skin lesions during the 30 min following intradermal injection of various doses of histamine and bradykinin was determined. Figure 1 shows that bradykinin was approximately two orders

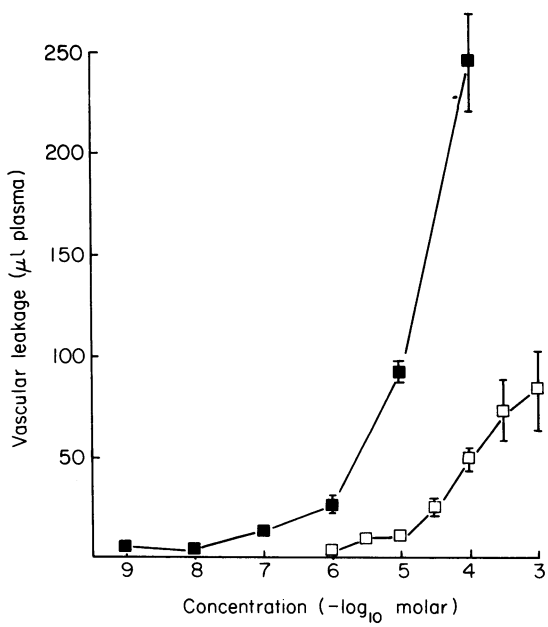

Figure 1. Dose respose of vascular leakage induced by histamine (□——□) and bradykinin (■——■). Vascular leakage of ^{125}I-HSA was determined for a period of 30 min after intradermal injection of histamine and bradykinin and is expressed as μl of plasma. Data points represent mean ± SEM of five lesions.

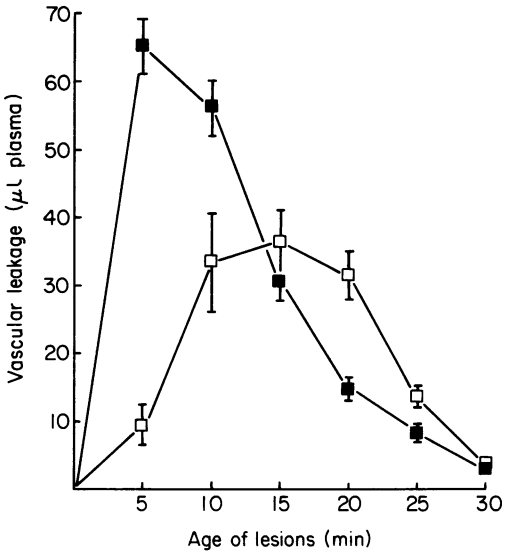

Figure 2. Kinetics of vascular leakage induced by 10^{-5} M bradykinin (■————■) and 10^{-3} M histamine (□————□). Sets of lesions were established every 5 min for 25 min and leakage of ^{125}I-HSA determined for the 5 min before killing.

of magnitude more potent in inducing enhanced vascular permeability than histamine and was able to induce much greater leakage than the highest doses of histamine used in these experiments. This conforms with previous observations on vascular leakage induced by these mediators in rabbits (Oyvin, Gaponiuk & Oyvin, 1967).

The kinetics of vascular leakage induced by 10^{-5} M bradykinin and 10^{-3} M histamine were studied in lesions up to 30 min old. A set of intradermal injections was established every 5 min, and ^{125}I-HSA was injected intravenously 5 min before killing. Figure 2 shows that bradykinin produced greatest leakage in lesions during the first 5 min, and leakage returned almost to baseline in lesions 25 min old. By comparison, vascular leakage induced by histamine was slower in onset, peaking in lesions 10–15 min old and declining to a low level by 30 min.

Kinetics of tachyphylaxis to histamine and bradykinin

Lesions were established with 10^{-5} M bradykinin and 10^{-3} M histamine up to 72 hr before restimulation with the same agent. Figure 3 compares leakage of plasma into lesions during the 15 min following restimulation

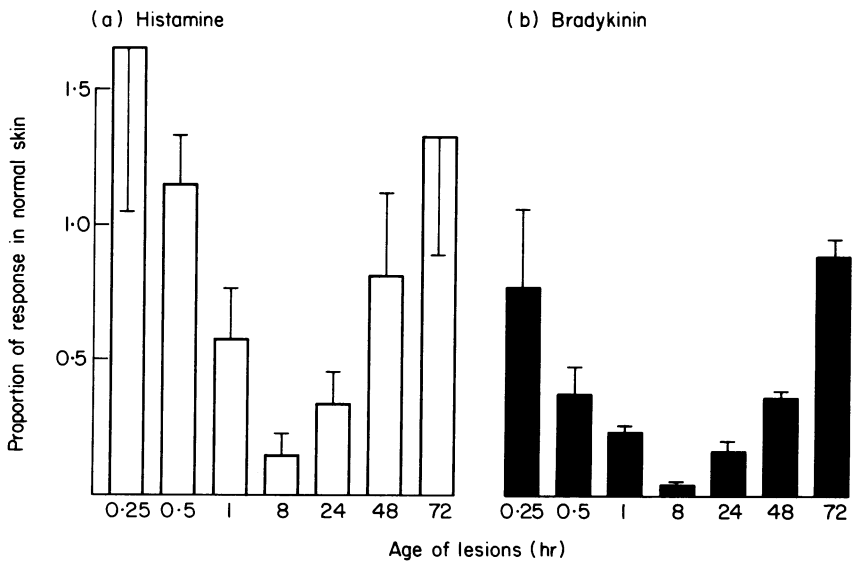

Figure 3. Kinetics of tachyphylaxis to (a) histamine and (b) bradykinin. Lesions were established at the indicated times with 10^{-3} M histamine and 10^{-5} M bradykinin, and leakage of ^{125}I-HSA determined for 15 min following restimulation with the same concentration of histamine or bradykinin. Data points are means ± SEM of results in three rabbits for each agent.

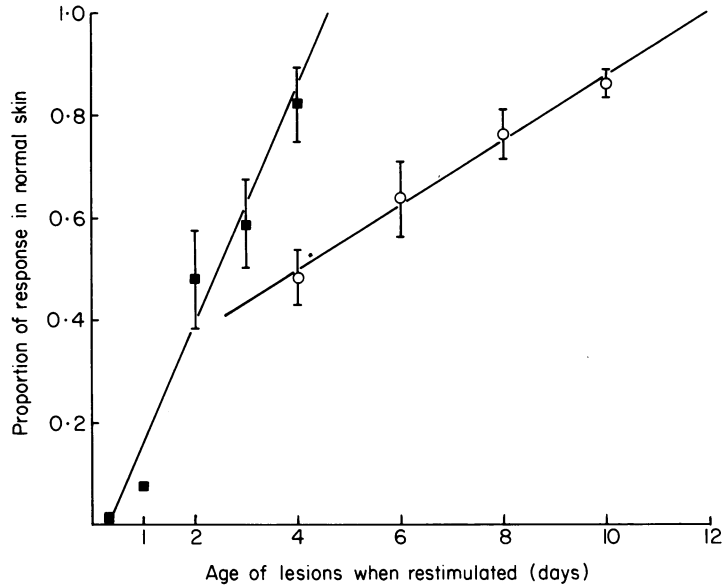

Figure 4. Kinetics of resensitization of lesions to histamine (■————■) and FMLP (○————○). Lesions were initiated with 10^{-3} M histamine or 10^{-5} M FMLP and restimulated with 10^{-4} M histamine and 10^{-6} FMLP, respectively. The responses measured were leakage of ^{125}I-HSA into lesions restimulated with histamine and accumulation of ^{51}Cr-neutrophils in lesions restimulated with FMLP. Linear estimation of the return of normal sensitivity yielded values of 4·7 days for histamine and 11·9 days for FMLP. Data points are means for two animals (histamine) and four animals (FMLP).

with leakage into adjacent normal skin sites stimulated for the first time. Lesions retained sensitivity to a second stimulus of histamine at 30 min. In contrast, vascular leakage was diminished when lesions 30 min old were restimulated with bradykinin. For both agents, lesions were profoundly desensitized at 8 hr and gradually regained sensitivity during the subsequent 4 days. In order to determine the kinetics of resensitization more accurately, lesions were initiated with $10^{-4.5}$ M bradykinin and 10^{-3} M histamine and restimulated with $10^{-5.5}$ M bradykinin and 10^{-4} M histamine, respectively. For each agent, there was a highly significant linear regression of sensitivity on time. Figure 4 plots the following regression equation for histamine:

$$y = 0{\cdot}227\,x - 0{\cdot}074\ (r^2 = 0{\cdot}965;\ \beta = 0,\ P < 0{\cdot}005).$$

By linear estimation, sensitivity to histamine returned in 4·7 days. Bradykinin yielded comparable results with the following regression equation:

$$y = 0{\cdot}229\,x - 0{\cdot}080\ (r^2 = 0{\cdot}972;\ \beta = 0,\ P < 0{\cdot}05)$$

and a linear estimate of return to normal sensitivity at 4·6 days.

Specificity of tachyphylaxis to histamine, bradykinin and FMLP

It has previously been shown that desensitization of neutrophil infiltration into skin lesions stimulated with chemotaxins is, in most instances, specific for each agent. For example, lesions initiated with FMLP, platelet-activating factor, zymosan-activated plasma, leukotriene B_4 and the chemotaxinigen endotoxin, were desensitized to homologous restimulation but not to restimulation with FMLP as a heterologous agent (Colditz & Movat, 1984a, b). Accordingly, the specificity of desensitization for vascular leakage to histamine, bradykinin and FMLP was studied in lesions initiated with $10^{-5.5}$ M bradykinin, 10^{-3} M histamine or 10^{-5} M FMLP 5 hr before restimulation. Table 1 shows the results of a typical experiment. Cross-desensitization occurred between histamine and bradykinin, and lesions initiated with a mixture of the two agents were profoundly desensitized to restimulation with the mixture. Vascular leakage in lesions initiated with FMLP and restimulated with the mixture of histamine and bradykinin was 32% of the response in skin sites stimulated with the mixture for

Table 1. Specificity of desensitization of skin lesions to bradykinin, histamine and FMLP

Initial stimulus: final stimulus*	Response†	Statistical significance‡
H:H	0·118±0·019	$P \ll 0.001$
B:H	0·608±0·069	$P < 0.01$
B:B	0·197±0·043	$P \ll 0.001$
H:B	0·346±0·045	$P < 0.001$
BH:BH	0·046±0·009	$P \ll 0.001$
FMLP:BH	0·321±0·045	$P \ll 0.001$
Saline:BH	0·927±0·123	NS§

* Initial stimulus was given 5 hr before the final stimulus. H, histamine 10^{-3} M; B, bradykinin $10^{-5.5}$ M; BH, histamine 10^{-3} M and bradykinin $10^{-5.5}$ M; FMLP, 10^{-5} M.

† Response is the quotient:

$$\frac{\text{c.p.m. in restimulated skin} - \text{background}}{\text{c.p.m. in skin given final stimulus only} - \text{background}}$$

and is the mean±standard error of the mean of five lesions.

‡ Statistical significance was determined by Student's *t*-test.

§ NS, not significant.

the first time. Lesions receiving an initial injection of saline responded normally to restimulation with the mixture of histamine and bradykinin.

The accumulation of neutrophils in lesions initiated with a mixture of bradykinin and histamine and restimulated with FMLP was 1·25±0·34 times that in normal skin sites stimulated with FMLP.

Kinetics of tachyphylaxis to FMLP

We have previously found that loss of sensitivity to FMLP and zymosan-activated plasma commences when lesions are 2–4 hr old and lasts at least 10 hr (Colditz & Movat, 1984a). Accordingly, the time course for recovery of sensitivity to FMLP was investigated over a longer period (4·3 days) for comparison with the results obtained with histamine and bradykinin. Figure 5 shows that lesions up to 4·3 days old responded to restimulation with FMLP with approximately 50% of the response in normal skin sites. In contrast, lesions initiated with FMLP displayed normal or greater than normal sensitivity to endotoxin throughout this period.

In a separate experiment, lesions were established with 10^{-5} M FMLP every 2 days for 10 days, and the neutrophil influx following restimulation with 10^{-6} M FMLP was determined. A highly significant linear regression of sensitivity on time occurred between 4 days and 10 days ($y = 0.064\ x + 0.241$; $r^2 = 0.990$; $\beta = 0$, $P < 0.01$) with an estimated return to normal sensitivity at 11·9 days (see Fig. 4). Slopes of the regression equations for vascular permeability agents and FMLP showed highly significant differences ($P \ll 0.001$).

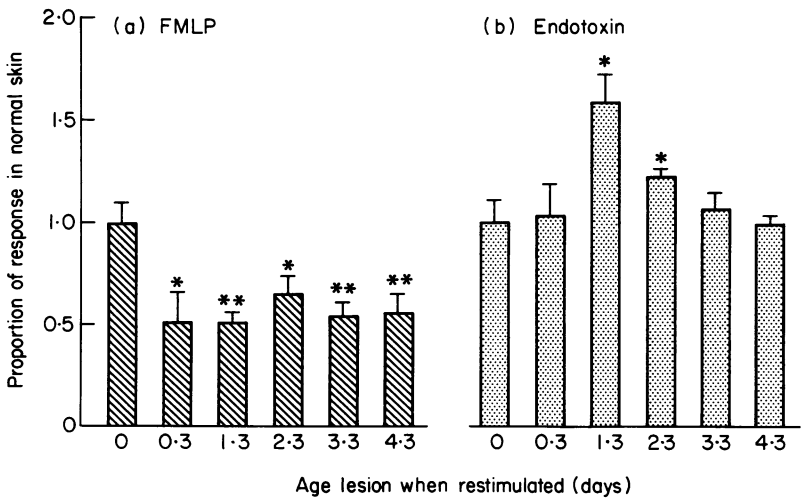

Figure 5. Neutrophil accumulation in lesions initiated with 10^{-6} M FMLP and restimulated with (a) 10^{-6} M FMLP or (b) 10^{-11} M endotoxin. Data points are means±SEM of results in four rabbits (FMLP) and three rabbits (endotoxin).

* $P < 0.05$ by Student's *t*-test.

** $P < 0.01$.

DISCUSSION

A role for histamine in inducing vascular leakage was proposed by Lewis & Grant (1924), who also observed a refractoriness of human skin to restimulation with histamine. In guinea-pigs, Miles & Miles (1952) found histamine-induced leakage, assessed by skin bluing, lasted around 15 min, and a refractory period peaked at 1–3 hr and declined after 4–5 hr. Oyvin *et al.* (1967) observed strong desensitization of rabbit skin to histamine but only modest desensitization to bradykinin. Similar results are reported in human skin where Greaves & Shuster (1967) observed tachyphylaxis to histamine but not bradykinin. In the present experiments, profound tachyphylaxis to both bradykinin and histamine was observed in rabbit skin, and resensitization to the two agents took approximately 4·7 days. The more sensitive estimates of vascular leakage provided by ^{125}I-HSA and variations between species may acount for differences between the present findings and previous reports.

Vascular leakage is potentiated by the hyperaemia induced by vasoactive agents such as prostacyclin and prostaglandin E (Johnston, Hay & Movat, 1976). In addition, several studies have shown a cascade effect of agents inducing vascular leakage. Fibrin peptides and bradykinin induce histamine release from mast cells (Johnsson & Erdos, 1973) and synthesis of prostacyclin (Alhenc-Gelas *et al.*, 1982). Conversely, hydrogen peroxide, which is generated by chemotaxin-stimulated neutrophils, has recently been shown to diminish the responsiveness of endothelial cells to histamine and bradykinin (Ager & Gordon, 1984). In *in vivo* studies, the permeability response to bradykinin in rabbit skin is diminished by antihistamines (Marceau, Knap & Regoli, 1981), and refractoriness to histamine has been observed in guinea-pig skin lesions initiated with bradykinin (Baumgarten, Melrose & Vagg, 1970). In the present experiments, considerable cross-desensitization occurred between histamine and bradykinin, and also was a sequel to FMLP-induced inflammation (Table 1). This is consistent with the liberation of additional mediators by the injected agents and is in marked contrast to the specificity of desensitization of lesions to chemotaxins (Colditz & Movat, 1984a, b). The greater desensitization induced by the mixture of histamine and bradykinin than by either agent alone suggests that desensitization may also occur to the additional mediators liberated by each agent. Comparison of the kinetics of vascular leakage and tachyphylaxis to bradykinin and histamine indicates that the principal vasoactivity of bradykinin is not dependent on histamine release. Tachyphylaxis and vascular permeability occurred more promptly in lesions stimulated with bradykinin than with histamine, thus, release of endogenous histamine is likely to play a minor role in bradykinin-induced vascular leakage.

Agents affecting vascular permeability have been found to potentiate the migration of leucocytes into lymph nodes (Moore, 1984) and into lesions of acute inflammation (Issekutz, 1981) and delayed-type hypersensitivity (Askenase, Metzler & Gershon, 1982). Two mechanisms have been proposed for this enhanced emigration of leucocytes. Firstly, hyperaemia following stimulation with these vasoactive agents may increase the delivery of leucocytes to the site of emigration (Hay & Hobbs, 1977; Issekutz, 1981; Issekutz & Movat, 1982). Secondly, contraction of endothelial cells may facilitate the passage of leucocytes between endothelial cells (Issekutz, 1981; Askenase *et al.*, 1982). It is noteworthy that desensitization to vascular permeability agents diminishes localization of leucocytes in delayed-type hypersensitivity lesions in mice (Askenase *et al.*, 1982). The transience of the permeability effect of histamine and bradykinin, however, argues against endothelial cell contraction being an important determinant of leucocyte emigration, which occurs over a much longer period than the permeability response. Moreover, in the present experiments, initiation of lesions with a mixture of histamine and bradykinin failed to diminish the neutrophil influx upon stimulation with FMLP. This suggests that sensitivity of the vascular bed to endogenous permeability agents does not limit the emigration of neutrophils during acute inflammation. The markedly different kinetics of resensitization to vascular permeability agents and FMLP indicate that these are independent events. Accordingly, it is unlikely that sensitivity to vascular permeability agents influences neutrophil accumulation in lesions during resensitization to the chemotaxin FMLP.

Several processes could contribute in tachyphylaxis to histamine and bradykinin. These include fatigue of the contractile response of endothelial cells, uncoupling of the receptor-effector pathway, and down-regulation of receptors for these mediators of vascular permeability. The concentrations of histamine and bradykinin used in these experiments were chosen to evoke a submaximal increase in vascular leakage. In kinetic studies of tachyphylaxis to histamine, restimulation of lesions 15 min old led to greater vascular

leakage than occurred in normal skin stimulated once (Fig. 3). This indicates that the leakage in restimulated lesions is not limited by extravascular tissue fluid pressure, nor by the potential of the vascular bed to undergo a second episode of vascular leakage. In addition, the failure of cross-desensitization to be as strong as homologous desensitization indicates that reactivity of the contractile apparatus of endothelial cells to permeability mediators does not limit extravasation of plasma during a second episode of increased vascular permeability. Diminished contractility of endothelial cells may, however, be a contributing factor in tachyphylaxis of the vascular leakage response to histamine and bradykinin. Receptor down-regulation can occur due to loss of receptors following their internalization, or through negative co-operativity between receptors when partial occupancy leads to decreased affinity of residual receptors for the agonist (reviewed by Catt *et al.*, 1979). Receptor number can return to normal by recycling of receptors, by deployment of internalized receptors or by synthesis of new receptors. Studies of endothelial cell turnover in normal vascular beds have yielded estimates of cell half-lives ranging from months to years (Engerman, Pfaffenbach & Davis, 1967; Wright, 1970; Sholley & Cotran, 1976), suggesting that resensitization does not require renewal of endothelial cells. Resensitization to vascular permeability agents, then, may require synthesis of new receptors or recoupling of the receptor-effector response.

Resensitization of lesions to the chemotaxin FMLP was slower and of later onset than that of histamine and bradykinin (Fig. 4). Previous experiments have shown that desensitization of inflammatory lesions to chemotaxins is not due to elaboration of an inhibitor of neutrophil migration, nor to decreased capacity of a lesion to support a second episode of infiltration by neutrophils (Colditz & Movat, 1984b). Inflammatory cells are cleared from a lesion within a few days by lymphatics and histiocytes (Clark, Clark & Rex, 1936), indicating that the immigrant cells are unlikely to regulate the sensitivity of a lesion to restimulation with the homologous chemotaxin. These considerations led to the suggestion that chemotaxin receptors regulate the accumulation of neutrophils in acute inflammatory lesions and may be located on endothelial cells of post-capillary venules. The present experiments indicate that, if endothelial cells are indeed the location of the regulatory chemotaxin receptors, then their expression is controlled independently of endothelial receptors for vascular permeability agents. The results are also consistent with the alternative proposal that a cell population remote from the vessel wall may bear the chemotaxin receptors which regulate neutrophil extravasation during acute inflammation (Colditz & Movat, 1984b).

ACKNOWLEDGMENTS

The author is supported by a CSIRO (Australia) postdoctoral fellowship. The assistance of Chris Scales and Chris Barnden is gratefully acknowledged.

REFERENCES

AGER A. & GORDON J.L. (1984) Differential effects of hydrogen peroxide on indices of endothelial cell function. *J. exp. Med.* **159,** 592.

ALHENC-GELAS F., TSAI S.J., CALLAHAN K.S., CAMPBELL W.B. & JOHNSON A.R. (1982) Stimulation of prostaglandin formation by vasoactive mediators in cultured human endothelial cells. *Prostaglandins*, **24,** 723.

ASKENASE P.W., METZLER C.M. & GERSON R.K. (1982) Localization of leucocytes in sites of delayed-type hypersensitivity and in lymph nodes: dependence on vasoactive amines. *Immunology*, **47,** 239.

BAUMGARTEN A., MELROSE G.J.H. & VAGG W.J. (1970) Interactions between histamine and bradykinin assessed by continuous recording of increased vascular permeability. *J. Physiol.* **208,** 669.

CATT K.J., HARWOOD J.P., AGUILERA G. & DUFAU M.L. (1979) Hormonal regulation of peptide receptors and target cell responses. *Nature (Lond.)*, **280,** 109.

CLARK E.R., CLARK E.L. & REX R.O. (1936) Observations on polymorphonuclear leukocytes in the living animal. *Am. J. Anat.* **59,** 123.

COLDITZ I.G. & MOVAT H.Z. (1984a) Chemotactic factor-specific desensitization of skin to infiltration by PMN-leukocytes. *Immunol. Lett.* **8,** 83.

COLDITZ I.G. & MOVAT H.Z. (1984b) Desensitization of acute inflammatory lesions to chemotaxins and endotoxin. *J. Immunol.* **133,** 2163.

COLDITZ I.G. & MOVAT H.Z. (1984c) Kinetics of neutrophil accumulation in acute inflammatory lesions induced by chemotaxins and chemotaxinigens. *J. Immunol.* **133,** 2169.

ENGERMAN R.L., PFAFFENBACH D. & DAVIS M.D. (1967) Cell turnover in capillaries. *Lab. Invest.* **17,** 738.

GREAVES M. & SHUSTER S. (1967) Responses of skin blood vessels to bradykinin, histamine and 5-hydroxytryptamine. *J. Physiol.* **193,** 255.

HAY J.B. & HOBBS B.B. (1977) The flow of blood to lymph nodes and its relation to lymphocyte traffic and the immune response. *J. exp. Med.* **145,** 31.

HELTIANU C., SIMIONESCU M. & SIMIONESCU N. (1982) Histamine receptors of the microvascular endothelium revealed *in situ* with a histamine-ferritin conjugate: characteristic high-affinity binding sites in venules. *J. Cell Biol.* **93,** 357.

ISSEKUTZ A.C. (1981) The effect of vasoactive agents on polymorphonuclear leukocyte emigration *in vivo*. *Lab. Invest.* **45,** 234.

ISSEKUTZ A.C. & MOVAT H.Z. (1982) The effect of vasodilator prostaglandins on polymorphonuclear leukocyte infiltration and vascular injury. *Am. J. Pathol.* **107,** 300.

JOHNSSON A.R. & ERDOS E.G. (1973) Release of histamine from mast cells by vasoactive peptides. *Proc. Soc. exp. Biol.* **142,** 1252.

JOHNSTON M.G., HAY J.B. & MOVAT H.Z. (1976) The modulation of enhanced vascular permeability by prostaglandins through alteration in blood flow (hyperemia). *Agents Actions*, **6,** 705.

LEWIS T. & GRANT R.T. (1924) Vascular reactions of the skin to injury. II. The liberation of a histamine-like substance in injured skin. *Heart*, **11,** 209.

MAJNO G., SHEA S.M. & LEVENTHAL M. (1969) Endothelial contraction induced by histamine-type mediators. *J. Cell Biol.* **42,** 647.

MARCEAU F., KNAP M. & REGOLI D. (1981) Pharmacological characterization of the vascular permeability enhancing effects of kinins in the rabbit skin. *Can. J. Physiol. Pharmacol.* **59,** 921.

MILES A.A. & MILES E.M. (1952) Vascular reactions to histamine, histamine-liberator and leukotaxine in the skin of guinea-pigs. *J. Physiol.* **118,** 228.

MOORE T.C. (1984) The modulation by prostaglandins of increases in lymphocyte traffic induced by bradykinin. *Immunology*, **51,** 455.

MOSES J.M., GESCHICKTER E.H. & EBERT R.H. (1968) Pathogenesis of inflammation. *Br. J. exp. Pathol.* **49,** 385.

OYVIN I.A., GAPONIUK P.Y. & OYVIN V.I. (1967) The effect of bradykinin on permeability of skin blood vessels. *Experientia*, **23,** 925.

REGOLI D. (1983) Pharmacology of bradykinin and related kinins. *Adv. exp. Med. Biol.* **156,** 569.

SHOLLEY M.M. & COTRAN R.S. (1976) Endothelial DNA synthesis in the microvasculature of rat skin during the hair growth cycle. *Am. J. Anat.* **147,** 243.

SIMIONESCU N., HELTLIANU C., ANTOHE F. & SIMIONESCU M. (1982) Endothelial cell receptors for histamine. *Ann. N.Y. Acad. Sci.* **401,** 132.

WILHELM D.L. (1973) Chemical mediators. In: *The Inflammatory Process* (eds B. W. Zweifach, L. Grant and R. T. McCluskey), 2nd edn, Vol. II, p. 251. Academic Press, New York.

WRIGHT H.P. (1970) Endothelial turnover. In: *Vascular Factors and Thrombosis*, (ed. F. Koller), p. 79. Schattauer Verlag, Stuttgart.