In Vivo Clearance and Tissue Distribution of C5a and C5a des Arginine Complement Fragments in Rabbits

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ABSTRACT We have previously shown a marked difference in the inflammatory response to human C5a or C5a des arginine (Arg) instilled in rabbit lungs. These studies raised the question of where C5a and C5a des Arg are processed in vivo and what role neutrophils may play in the tissue distribution of these two mediators. After intravenous injection of purified, biologically active ^{125}I-C5a or ^{125}I-C5a des Arg, adult rabbits were serially bled and then killed at various time intervals. Although >50% of the injected radioactivity was cleared from the circulation within 2 min for both mediators, C5a des Arg persisted in the circulation longer than C5a. C5a instillation caused an acute neutropenia, whereas C5a des Arg caused a less severe and more prolonged neutropenia, preceding a neutrophilic response observed with both mediators. Clearance of the mediators was primarily seen in the highly vascularized organs: the lung, spleen, liver, and kidney. A time-dependent accumulation was seen initially in the lung, followed by the spleen, liver, and kidney. Histologic examination showed a marked increase in the number of neutrophils within the lung and spleen. Depletion of circulating neutrophils by nitrogen mustard pretreatment of rabbits showed no change in the amount of labeled mediator bound in the lung, whereas splenic accumulation was dependent on the presence of neutrophils. These results indicate that C5a and C5a des Arg are rapidly removed from the circulation by specific accumulation in vascularized tissues. Clearance by the lung was not affected by neutrophil depletion, whereas clearance by the spleen was dependent on neutrophils. These experiments further suggest that there are neutrophil-dependent and neutrophil-independent mechanisms involved in the removal of C5a and C5a des Arg from the circulation and that binding of C5 fragments in the pulmonary vasculature may precede and then induce neutrophil sequestration.

INTRODUCTION

Activation of the human complement system by either the classical or alternative pathways results in the generation of the biologically active fragment C5a. The presence of the naturally occurring serum enzyme, carboxypeptidase N, results in the rapid cleavage of the COOH-terminal arginine from C5a forming C5a des arginine (Arg) (1). Previous reports have shown that although human C5a des Arg shows little, if any, activity as an anaphylatoxin (2), it has intrinsic properties as an inflammatory mediator (3–5). Like C5a, C5a des Arg is capable of stimulating neutrophils to release lysosomal enzymes, to migrate in a directed fashion (chemotaxis), and to generate superoxide anion. Our previous studies showed a differential ability of C5a and C5a des Arg to induce inflammation when instilled in rabbit lungs (6). These studies raised the question of how C5a and C5a des Arg are processed in vivo. C5a has been suggested to have a systemic effect on numbers of circulating neutrophils. Intravascular instillation of cobra venom factor (to activate the alternative pathway) or C5 fragments has been shown to cause a transient neutropenia followed by neutrophilia (7, 8). However, the effect of purified C5a or C5a des Arg on circulating neutrophil levels has not yet been reported. It has also been suggested that since C5a and C5a des Arg bind to neutrophils (9), these inflammatory mediators are distributed in the body as these cells are transported through the vasculature. This current study reports on the kinetics of clearance from the circulation as well as tissue distribution of radiolabeled C5a and C5a des Arg. The effect the C5-

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derived peptides have on numbers of circulating neutrophils, as well as the role neutrophils play in the tissue distribution of labeled C5a and C5a des Arg, were also examined.

METHODS

Animals. New Zealand White rabbits (2.5–3.5 kg) were obtained from L.I.T. Rabbitry, Whitehall, MT, and were maintained on a standard pelleted diet and water. 48 h before injection of labeled C5-fragments, the rabbits were given a potassium iodide-sodium chloride solution (0.03 and 0.1 mg/ml, respectively) instead of water.

Human C5a and C5a des Arg. Human C5a and C5a des Arg were prepared as previously described (5). The isolated C5-derived peptides were shown to be homogeneous by acid and SDS polyacrylamide gel electrophoresis. Both peptides were radiolabeled with ^{125}I to 0.8–7.0 μCi/μg sp act using a solid-phase lactoperoxidase-glucose oxidase procedure (Bio-Rad Laboratories, Richmond, CA). The radioactive label was shown to be >95% TCA precipitable. Following the labeling procedure and exhaustive dialysis to remove unbound ^{125}I, some radiolabeled preparations were passed over an affinity column (1 × 3 cm) containing rabbit anti-human C5a coupled to Sepharose-4B. As had been described by Chenoweth and Hugli (4), biologically inactive and antigenically altered C5a passed through the column, whereas biologically active labeled C5a remained bound to the affinity beads. The bound C5a was eluted from the column with 0.1 M glycine, pH 3.0 containing 1.0 M NaCl. Eluted fractions (1.0 ml) containing C5a were pooled and dialyzed vs. water followed by phosphate-buffered (pH 7.2) saline. The column purified ^{125}I-C5a or ^{125}I-C5a des Arg was stored at 0°C in wet ice and used within 48 h.

Cobra venom factor. Cobra venom (Bioactive Inc., Arlington, VA) was subjected to DEAE-Sephadex chromatography as described by Cochrane and Muller-Eberhard (10) and treated with *p*-bromophenacyl bromide to remove any trace phospholipase A_2 contamination (11). The biologic activity of the cobra venom factor (CVF)[1] was hemolytically assayed and titered (10). The CVF was used to systemically generate C5a in vivo by the intravascular instillation of 300 U/animal.

Rabbit hemolytic complement assays. Hemolytic titers of whole rabbit complement (CH_{50}) and rabbit C5 ($C5H_{50}$) were assayed by a modification of the method of Giclas et al. (12), as previously described (13).

Determination of blood volume with serum albumin. Rabbit serum albumin (RSA) was isolated as previously described (13). It was radiolabeled with ^{131}I to a specific activity of 3–5 μCi/μg using the lactoperoxidase-glucose oxidase procedure (Bio-Rad Laboratories). To determine tissue blood volume, 3 μCi of ^{131}I-RSA in 1 ml were injected via a peripheral ear vein 5 min before killing of the rabbit. Samples of tissues were then weighed and placed into plastic counting vials and the amount of radioactivity present determined in a gamma counter (Beckman Instruments, Inc., Irvine, CA). Corrections for the blood content of the various tissues was made by calculating the counts per minute per milliliter blood and counts per minute per gram of tissue of ^{131}I-RSA.

Neutrophil-depleted animals. To determine the role circulating neutrophils had on the distribution of labeled C5a, some animals were treated with nitrogen mustard (mechlorethamine HCl) (Merck, Sharp and Dohme, West Point, PA). These animals received 1.75 mg/kg body wt i.v. 72 h before the injection of the labeled peptides. Blood counts at the time of the injection showed <1% circulating neutrophils. Histologic examination also showed a marked depletion of neutrophils within the tissues.

Blood cell measurements. Determination of the numbers of circulating leukocytes was performed in triplicate using a Coulter model ZBI counter (Coulter Diagnostics, Hialeah, FL). Differential counts were made on air-dried microscope slide smears stained with Wright's stain. The numbers of neutrophils were expressed as the percentage of initial value and are averages of at least three animals in each group.

Experimental procedure. Initially, rabbits were secured in a rabbit restrainer and a Teflon arterial cannula (Intracath, The Deseret Co., Sandy, UT) placed in the middle ear artery for collection of blood samples. Later experiments were performed using a surgically implanted catheter in the femoral artery for blood collection. In either case, sterile, pyrogen-free saline was infused at 4 ml/h to maintain an open line and to maintain the animal's intravascular volume. A 2–20-μg bolus of C5a or C5a des Arg containing 2–5 μCi of ^{125}I in 1 ml of sterile, pyrogen-free saline was injected in the peripheral ear vein. Blood samples were obtained at various intervals over 200 min after injection of the C5-derived peptides to determine kinetics of radioactive clearance and to determine the numbers of circulating neutrophils.

Tissue distribution of labeled C5a or C5a des Arg was determined in animals killed at 4, 30, or 120 min after injection of the peptides. Whole organs or samples of tissues were cleared of fat and connective tissue, weighed, and along with samples of blood, urine, and bile, counted for the presence of both ^{125}I-C5 fragment and ^{131}I-RSA in a dual channel gamma counter. The total micrograms of C5a or C5a des Arg per gram of tissue was calculated based on the ^{125}I counts per minute per gram tissue corrected for blood volume and the specific activity of the injected fragment.

Normalization of C5a (C5a des Arg) tissue content. The amount of C5a or C5a des Arg in each tissue is presented as a normalized value R. R was calculated by the following formula: R = micrograms of C5a in the tissue per gram of tissue/micrograms of C5a injected per gram of total body weight. This calculation has been used by several investigators to compare these type of data (14, 15). These normalized values were used to compare C5a accumulation since they take into account rabbit-to-rabbit variation in tissue and body weight as well as differences in specific activities of the radiolabeled peptides. The data are expressed as the mean±SEM of R for three rabbits at a given time after injection. Due to the variability of urine volume in the bladder at the time of death, these data were calculated as R° = total micrograms of C5a in urine divided by the micrograms of C5a injected per gram of body weight.

Molecular weight determinations. Attempts to estimate the molecular weight of radiolabeled fragments found in rabbit urine were made by ultrafiltration dialysis and autoradiography of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis after removal of urine sediment by centrifugation (1,500 *g* for 15 min). Aliquots of urine (10.0 ml) were placed into low molecular weight cutoff (3,500) dialysis tubing (Spectra/Por 3, Spectrum Medical Industries, Inc., Los Angeles, CA) and dialyzed exhaustively vs. phosphate-buffered (pH 7.2) saline. The amount of radioactivity present in the dialysate and dialysis tubing was determined in a gamma counter and compared to the initial amount of radioactivity. Urine was also electrophoresed on

[1] *Abbreviations used in this paper*: CVF, cobra venom factor; RSA, rabbit serum albumin.

10–20% linear gradient polyacrylamide gels in the presence of 0.1% SDS with or without reduction by prior incubation with 2.5% 2-mercaptoethanol (16). Molecular weight markers consisting of cytochrome *c* (12,400), insulin (5,600), and the α- and β-chains of reduced insulin (3,300 and 2,300, respectively) were included in each experiment. After staining and drying, the gels were exposed to LKB H film (LKB Instruments, Rockville, MD) at −70°C for 7–10 d. Exposed film was developed according to the manufacturer's instructions.

RESULTS

Clearance of C5a and C5a des Arg from the circulation. The kinetics of clearance of C5a and C5a des Arg from the circulation of rabbits were determined using radiolabeled peptides. As shown in Fig. 1, both C5-derived mediators were rapidly cleared from the circulation. Greater than 50% of the radiolabeled fragments were removed within 2 min after injection into the vasculature. By 30 min, <20% of the label was found in the circulation. This amount of radioactivity remained constant up to 4 h (data not shown). It was possible that the intravenous administration of C5a may have caused an alteration of the vascular volume that would affect the rate of clearance of C5a. The absence of change in the rate of clearance of ^{131}I-RSA given 30 min before the infusion of C5a showed that there was no significant change in vascular volume over 120 min (data not shown). No discernable difference in clearance of either label was observed between whole blood and plasma. Although the differences in clearance between C5a des Arg and C5a were not large, they were very reproducible and statistically significant ($P < 0.05$, Student's *t* test) with the clearance of C5a des Arg being less rapid.

This result may explain in part the differences C5a and C5a des Arg have on the levels of circulating neutrophils (Fig. 2). As had been shown by other investigators, instillation of a single bolus of human C5a into the circulation results in a rapid but transient neutropenia (<1% circulating neutrophils at 1 min) followed by a neutrophilia. The C5a-induced neutropenia was over by 5 min. Injection of a single bolus of the same amount of C5a des Arg, on the other hand, produced a more prolonged neutropenia (1–30 min) that was less marked (50% decline) than that produced by C5a. A neutrophilia was also seen after C5a des Arg administration. Although there were differences in the kinetics and the degree of neutropenia seen after intravenous C5a des Arg vs C5a, both mediators appeared equally potent in producing the neutrophilia.

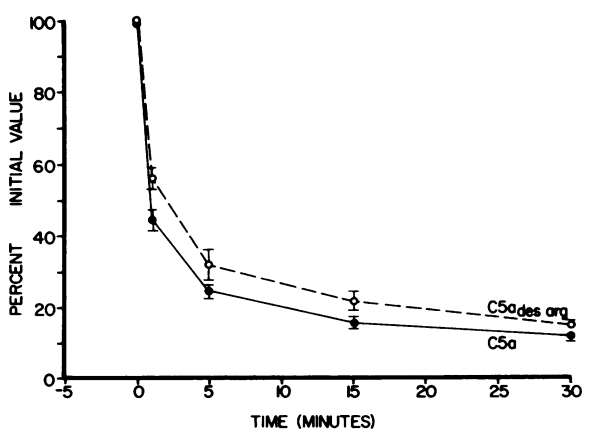

FIGURE 1 Clearance of C5a and C5a des Arg from the circulation. Rabbits received either ^{125}I-C5a or ^{125}I-C5a des Arg (2–20 μg) intravenously. The data are expressed as the mean %±SEM of the initial C5a (C5a des Arg) input. There were six animals in each group.

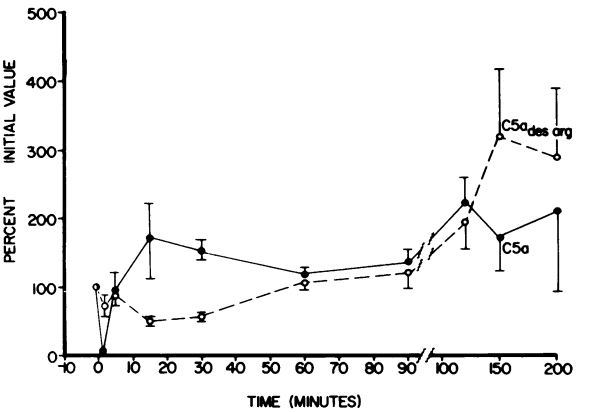

FIGURE 2 Effect of intravenous C5a and C5a des Arg on circulating neutrophil counts. Rabbits received 2–20 μg of C5a or C5a des Arg intravenously. The data are expressed as the percent base-line value±SEM for three animals in each group.

Tissue distribution of intravascular C5a and C5a des Arg. As shown in Table I, accumulation of the labeled mediators was primarily seen in the highly vascularized organs of the lung, liver, kidney, and

TABLE I
Tissue Distribution of ^{125}I-C5a 30 Min Postintravenous Injection

	R*		R
Lung	9.67	Choroid plexus	0.01
Liver	3.97	Diaphragm	0.04
Kidney	20.29	Gall bladder	0.29
Spleen	19.79	Gut	0.09
Abdominal muscle	0.03	Heart	0.15
Adrenal gland	0.13	Mesenteric lymph node	0.05
Aorta	0.01	Skin	0.24

* $R = \frac{\mu g\ C5a/g\ tissue}{\mu g\ C5a\ injected/g\ body\ wt}$.

spleen (R = 3.97–20.29). Other examined tissues showed an R value of <0.3. At no time point were significant differences between C5a and C5a des Arg noted in the tissue accumulation of radioactive label. 65.4±3.2% (mean±SEM, n = 34) of the injected material was accounted for by summing the radioactivity recovered in all of the tissues examined. A degree of specificity of accumulation within vascularized tissues was shown by the absence of labeled fragments in the choroid plexus, another very highly vascularized tissue. At early time points, the lung was the most effective site of accumulation of these mediators as shown in Fig. 3. The R value went from 10.74±1.08 (mean±SEM) at 4 min to 3.30±0.48 by 120 min. Low levels of accumulation by the liver were seen at all time points examined (R = 2.82±0.44 to 1.58±0.18). This was followed sequentially by accumulation in the spleen and kidney. The values for the spleen varied from R = 7.05±3.56 at 4 min to a plateau R value of 19.79±1.33, while the kidney peaked at 30 min (R = 20.29±4.88). Corresponding to a decrease of labeled peptides in the kidney was the accumulation of label in the urine (R° = 82±2 at 4 min; 294±74 at 30 min; and 1,483±275 at 120 min).

The radioactivity found in the urine represented breakdown products of the labeled peptides as only 8.1±1.3% (mean±SEM, n = 8) of the counts were TCA precipitable. After centrifugation of the urine, 15–20% of the total radioactivity was pelleted with the sediment. Further evidence that the radiolabeled peptides had been catabolized into low molecular weight peptides (<3,500), was obtained from ultrafiltration dialysis of the urine as >90% (range 91–95%) of the radiolabel found in the urine passed into the dialysis buffer. Attempts to determine the exact size of the C5a breakdown products in the urine by SDS-polyacrylamide gel electrophoresis were unsuccessful. Incubation of the urine with SDS resulted in the formation of a gel that was incompletely solubilized upon addition of small quantities of buffer. Further complications involved the presence of large amounts of salt in the urine, which totally distorted the electrophoretic gel pattern. Attempts to remove the salt by dialysis or gel filtration resulted in major losses of radioactivity.

The initial accumulation of label in the lung could have been due to binding of the C5-derived peptides in the first vascular bed encountered after injection into the ear vein. This possibility was investigated by instillation of labeled C5a into the femoral artery of rabbits. Accumulation of C5a in the various tissues did not vary from that seen when it was instilled into the ear vein (data not shown). This further suggested a degree of specificity for accumulation of labeled C5a in the various tissues.

Neutrophil-dependent and independent mechanisms of C5a(C5a des Arg) clearance. Several investigators have shown binding of C5a and C5a des Arg to neutrophils (9, 17). Histologic examination showed a marked increase in the number of neutrophils within the lung and spleen following the instillation of C5a or C5a des Arg. Therefore, the question arose as to whether the rapid clearance of C5a and C5a des Arg from the circulation was due to binding to neutrophils and subsequent sequestration of the neutrophils in the vascularized tissues. In order to answer this question, normal rabbits or rabbits pretreated with nitrogen mustard to deplete circulating neutrophils, were given radiolabeled C5a or C5a des Arg. The tissues were then examined at various time points for the accumulation of radiolabel. As seen in Fig. 3A, clearance of the labeled mediators through the spleen was markedly dependent on the presence of circulating neutrophils at all examined time points (i.e., neutrophil depletion resulted in a decrease of up to 90% of tissue-bound label). Neutrophil depletion resulted in ~30–50% reduction in accumulation of labeled peptides in the

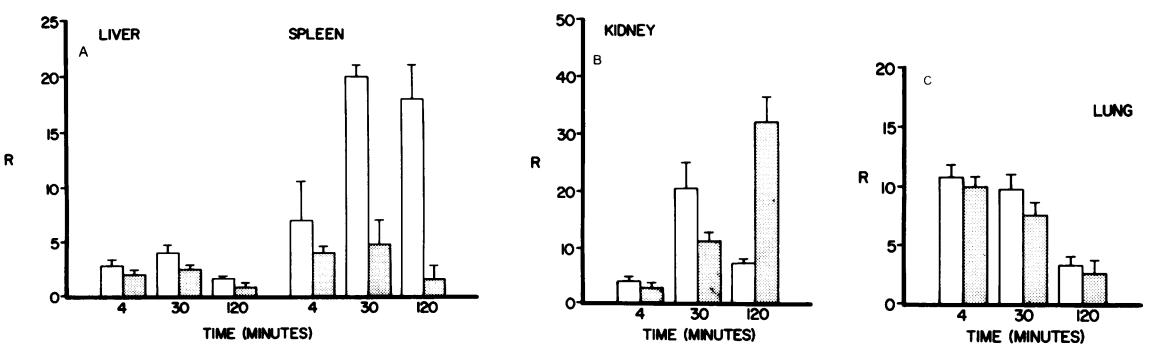

FIGURE 3 Effect of neutrophil-depletion on ^{125}I-C5a distribution. Normal rabbits, □, or rabbits given 1.75 mg/kg nitrogen mustard i.v. 72 h prior to study to deplete circulating neutrophils, ▨, were injected with 5 μg ^{125}I-C5a. The data are expressed as the ratio, R (mean±SEM), for three animals at each time point. Similar data were observed for C5a des Arg. A. Liver and spleen; B. Kidney; C. Lung.

liver. Clearance through the kidney (Fig. 3B), showed a biphasic response to neutrophil depletion; at early time points, it too showed a neutrophil dependent response for accumulation of the labeled fragments. However, at the later time point (120 min) an increased amount of label was seen in the neutrophil-depleted animals.

Unlike the neutrophil-dependent accumulation of ^{125}I-C5a(C5a des Arg) in the liver, spleen, and kidney, a neutrophil-independent mechanism of accumulation of labeled peptides in the lung was observed at all examined time points (Fig. 3C). Therefore, neutrophils do not appear to carry C5 fragments to the lung where the radioactivity subsequently accumulates.

The accumulation of radioactivity in the urine at 120 min was also dependent on circulating neutrophils. As shown in Fig. 4, normal rabbits had 32.2±3.4% (mean±SEM, $n = 3$) of the injected label vs. 17.3±2.5% for neutrophil-depleted animals ($P < 0.05$). No significant differences between the two groups of animals were seen at the earlier time periods. As had been observed in the urine of normal rabbits, <10% of the radioactivity was TCA precipitable in the urine of the neutrophil-depleted animals.

C5a(C5a des Arg) accumulation in tissues is a specific process. It was conceivable that the data might be explained in part by nonspecific binding of labeled fragments in highly vascularized tissues. Neutrophil-depleted rabbits were pretreated 24 h before instillation of ^{125}I-C5a with 300 U of CVF, which reduced CH_{50} and $C5H_{50}$ levels to 8–16% of initial values and may be presumed to cause in vivo generation of the chemotactic fragments C5a and C5a des Arg. After killing the animals, tissue distribution of radioactive label was determined as before. If the binding of the label was nonspecific, no decrease in tissue binding would be expected. If, on the other hand, binding was specific, a decrease in binding of the labeled peptides due to occupancy (or down regulation) of receptors by C5a des Arg in vivo by the previously generated C5 fragments would be expected. As seen in Table II, in vivo generation of chemotactic factors suppressed the binding of ^{125}I-C5a by as much as 64% as compared with the non-CVF-treated animals. Thus, specific binding of labeled C5a to the tissues was indicated. A concomitant increase in radioactivity was seen in the circulation of the animals pretreated with CVF.

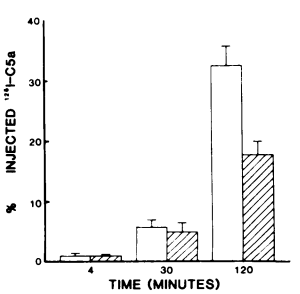

FIGURE 4 Effect of neutrophil-depletion of ^{125}I-C5a accumulation in the urine. Experimental conditions were the same as described in Fig. 3. The data are presented as the percent of injected label (mean±SEM), of three animals in each group. □, normal rabbits; ▨, neutrophil-depleted.

TABLE II
^{125}I-C5a Binding to Tissues in Rabbits Exposed to In Vivo Chemotactic Factors°

Tissue	Percentage
Lung	−41.6
Liver	−39.7
Kidney	−64.0
Spleen	−46.7
Blood	+52.3

° Neutrophil-depleted rabbits were given 300 U CVF 24 h earlier.

DISCUSSION

Highly purified, biologically active, radiolabeled C5a and C5a des Arg were used to study the clearance and tissue distribution of the C5-derived peptides in the rabbit.

Clearance of C5a and C5a des Arg from the blood. This study has demonstrated that both C5a and C5a des Arg are rapidly removed from the circulation of rabbits. Both molecules showed an initial rapid clearance of <2 min from the circulation. This compares with a previous report of 3 min as measured by aggregometry (18). The discrepancies in these data may be explained by procedural differences. Despite the initial rapid clearance of both of these mediators, C5a des Arg persisted in the circulation for a longer period of time than C5a, which may be related to differences in binding affinity between C5a and C5a des Arg as has been shown for neutrophil binding by these molecules (9).

As had been previously shown for CVF (7), human C5-fragments (8), and zymosan-activated plasma (8, 19, 20), instillation of C5a into the circulation resulted in a rapid, and near total, neutropenia followed by a neutrophilia. Injection of C5a des Arg, on the other hand, produced a more prolonged neutropenia than C5a before producing a neutrophilia. These observations are consistent with the fact that C5a des Arg binds less avidly to neutrophils than C5a (9, 17). Therefore, C5a des Arg remains in the circulation longer and its biological activity may persist longer than C5a. The degree of neutrophilia, seen after both mediators was variable from animal to animal, but was consistently elevated above the initial values. Intravascular instillation of partially purified rabbit C5a and C5a

des Arg resulted in similar effects on the levels of circulating neutrophils (21). However, the absence of purified rabbit C5a precluded its use in these current experiments. The mechanism of action of how these C5-derived peptides cause neutrophilia remains unknown. Because purified molecules were injected into the rabbits, the leukocytosis-inducing activity of C3e (22, 23) would not be likely. Whether C5a and C5a des Arg (or a split-product of these molecules) act similarly to C3e remains a possibility. In any event, the neutrophilia does not appear to be due to endotoxin-contamination of the C5-derived peptides. The amount of endotoxin present (<100 ng/ml as detected by a Limulus assay [Associates of Cape Cod, Woods Hole, MA]) was much less than that shown to cause leukocytosis (24, 25).

Tissue localization of C5a and C5a des Arg. The tissue distribution of C5a and C5a des Arg was primarily confined to the highly vascularized tissues: the lung, liver, spleen, and kidney. Other highly vascularized tissues, such as the choroid plexus, showed no greater accumulation of radiolabeled peptides than skin or muscle. Localization of the labeled peptides within the lung was not dependent on location of the vascular bed through which the mediator first passed. Instillation of C5a into a femoral artery resulted in the same pattern of labeled mediator accumulation as when it was given via the marginal ear vein. This finding contrasts with that recently described for the clearance of thrombin from the circulation in rabbits (26) in which the clearance was markedly dependent on the proximity of the vascular bed to the site of injection.

The specificity of C5a and C5a des Arg binding to vascularized tissues was further shown by in vivo generation of C5a and C5a des Arg by administration of CVF before instillation of labeled C5-peptides. All of the major organs where C5a and C5a des Arg were found to accumulate, now showed a decrease of 40% or greater in the amount of accumulated radioactivity with a concomitant increase in the amount of radioactivity found in the circulation. This suggested that these tissues have specific binding sites for C5-derived peptides.

Role of neutrophils in the tissue distribution of C5a and C5a des Arg. At least two mechanisms appear to be involved in the tissue distribution of C5a and C5a des Arg found within the circulation. Accumulation of radiolabeled peptides in the spleen was markedly neutrophil-dependent. This suggested that C5a and C5a des Arg bind to neutrophils, which are then trapped within the vasculature of the spleen. Such a mechanism is similar to that suggested by Hammerschmidt and co-workers (27) in which neutrophils are thought to be first aggregated by C5a and then become trapped in the microvasculature. However, this mechanism of action does not appear to be true for the lung in which a neutrophil-independent mechanism of C5a and C5a des Arg accumulation was seen.

One cautionary note is that we do not know the possible effect of other leukocytes (e.g., monocytes) in carrying C5 fragments to the lung. Nor do we know for certain the nitrogen mustard treatment results in complete clearance of all marginated neutrophils in the lung by 72 h. However, the neutropenia in the nitrogen mustard-treated rabbits persisted in spite of intravascular instillation of C5a or C5a des Arg (unpublished observation). It appeared likely, however, that C5a and C5a des Arg bind in the pulmonary vascular walls. Circulating neutrophils might then become attached to the endothelium as blood passes through the microvasculature. In vitro experiments by Hoover and co-workers (28) have shown that pretreatment of cultured endothelial cells with C5 fragments or *N*-formyl-methionine-leucine-phenylalanine will enhance the binding of neutrophils. Our own experiments, however, have so far been unable to reproduce these observations (29). Attempts to demonstrate direct binding of radiolabeled C5a to the endothelium have been marginally successful at best. Autoradiography of rabbit lung tissue exposed to intravascular ^{125}I-C5a has not shown specific accumulation anywhere, apparently because the specific activity of the labeled peptides was not high enough.

Accumulation of radiolabeled peptides in the kidney in neutrophil-depleted animals showed a delayed response compared with neutrophil-sufficient animals. The decreased levels of label at the early time points (4 and 30 min) probably reflected less C5a (C5a des Arg) being processed or carried to the kidney in the absence of circulating neutrophils. On the other hand, the increased clearance of label at the later time point (120 min) could reflect the absence of C5a binding to circulating neutrophils found in normal animals, therefore resulting in more cleared from the blood by the kidney. The appearance of label in the kidney was reflected by the amount of radioactivity found in the urine. It appeared that the presence of neutrophils within the circulation may play a major role in the processing of C5a (C5a des Arg) as has been reported in vitro (30). In the absence of neutrophils, removal of the labeled peptides still proceeds, but at a slower rate. The radioactivity found in the urine of both groups of animals represented breakdown products of the labeled peptides as determined by TCA precipitation and ultrafiltration dialysis.

Several unresolved questions regarding whether the endothelium plays an active role along with inflammatory cells during inflammation and whether soluble mediators such as complement-derived fragments alter endothelial cell function remain to be investigated. Answers to these questions should give further insight

into the basic mechanisms of inflammation within the vasculature.

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