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Reproduction of the Eosinopenia of Acute Infection by Passive Transfer of a Material Obtained from Inflammatory Exudate

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Studies of the eosinopenic effect of acute inflammation were conducted in mice previously rendered eosinophilic with trichinosis. Exudate removed from a pneumococcal abscess contained material (eosinopenic factor [EF]) capable of causing eosinopenia of 4- to 24-h duration when injected intraperitoneally into eosinophilic mice. The material passed through a 0.45-μm filter, but was retained by a dialysis membrane capable of retaining protein molecules of greater than approximately 30,000 molecular weight. EF was soluble in 7% perchloric acid, was not destroyed by pneumococcal proteolytic enzymes in the presence of Trasylol, but was inactivated by heating to 56°C for 30 min. EF was detectable in the exudate after 10 h and had reached its highest concentration after 20 h. When the effect of EF was expressed as a percent suppression of control eosinophil levels, there was a geometric dose response. Eosinopenia could not be ascribed to steroids present in the preparation, and the EF was effective in adrenalectomized animals. Eosinopenia was not induced by transfer of similarly treated heat-killed pneumococci, pneumococcal culture filtrate, or normal serum. The eosinopenia of acute infection may be the direct effect of a substance present at the site of acute inflammation.

An abrupt reduction in the number of circulating eosinophils occurs with the onset of many acute infections (10, 12, 14). The mechanism of this eosinopenia of acute infection has not been proven, although it has been assumed to reflect a response to stress that causes adrenal corticosteroid release (12). Recent studies have reexamined the phenomenon in a murine model (1, 2, 8). Mice were first rendered eosinophilic by infection with trichinosis. The suppression of this eosinophilia by acute inflammatory stimuli was then examined. Eosinopenia accompanied induction of acute inflammation by bacterial, viral, or chemical irritant stimuli (2). The sudden drop in eosinophil levels occurred independently of adrenal stimulation and in adrenalectomized animals (1). Eosinophils rapidly accumulated in the vicinity of acute inflammation, apparently in sufficient numbers to account for the initial eosinopenic response (2). These observations suggested that the eosinopenia might be induced by a substance in the inflammatory exudate. The present study examined this possibility. An extract of an inflammatory exudate has been shown to produce an eosinopenic response when injected intraperitoneally into mice with trichinosis.

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MATERIALS AND METHODS

Unless stated, the experimental methods were the same as described previously (1, 2).

Basic experimental design. Eosinophilia was produced by administering muscle-stage larvae of *Trichinella spiralis* to C3H/mg mice. Trichinosis in mice produces two peaks of eosinophilia, the first beginning 9 to 10 days after inoculation and coinciding with the migration of larvae from the intestinal tract, and the second beginning 21 to 22 days after inoculation, during the period of larval encystation in striated muscle. Eosinophil counts were obtained on day 22 or 23 of trichinosis. After verification that the eosinophilic response was underway, the animals were separated into equivalent groups by their eosinophil levels. Material to be tested was injected intraperitoneally at 9:00 p.m. on day 22 or 23 of trichinosis. Eosinophil counts of experimental and control groups were then determined between 9:00 and 11:00 a.m. on days 23 and 24 at the expected peak of eosinophilia.

Preparation of inflammatory exudate. Inflammatory exudate was obtained by lavage of a subcutaneous pneumococcal abscess. Production of a 2.5-ml subcutaneous air pouch and inoculation with 2×10^8 type 3 pneumococci were performed as described previously (1). This produced an acute exudative response within the subcutaneous air pouch. After 20 h, the mice were sacrificed by cervical dislocation, hair over the area was shaved, the abscess was opened, and the exudate was removed with a Pasteur pipette. Approximately 0.1 ml of exudate could be removed from each abscess. In the initial experi-

ments, the abscess was then lavaged with 0.5 ml of pH 7.4 phosphate-buffered saline (PBS) (Dulbecco A, Oxoid, London) containing penicillin G (1,200 U/ml). The exudates and lavage solutions were pooled and held at 4°C. They were centrifuged at 1,500 × *g* for 10 min and then at 17,000 × *g* for 30 min, and passed through an 0.45-μm filter (Millipore, Corp., Bedford, Mass.). To assure prevention of transfer of active pneumococcal infection, all recipients were routinely given 4 mg of procaine penicillin G subcutaneously. Intraperitoneal inoculation of one encapsulated pneumococcus into a mouse is sufficient to cause a lethal infection (11); none of the mice died after the transfer experiments.

In the initial experiments, mice were injected with 0.6 ml of the pooled material described above. The biological activity of such a preparation varied, depending on the age of the abscess from which it had been obtained. The quantity of exudate material transferred will be espressed as the "abscess dose," one abscess dose being the amount of material obtained from the lavage of one pneumococcal abscess. Unless specified otherwise, all experiments involved transfer to each recipient of one abscess dose of material obtained from a pneumococcal abscess of 18- to 20-h duration.

In all experiments, control animals received subcutaneous procaine penicillin G and an intraperitoneal injection of PBS that had been treated in a manner identical to that of the inflammatory exudate material.

Preparation of eosinopenic factor (EF). In experiments on heat inactivation, dose response, effect in adrenalectomized animals, and effect on neutrophils and lymphocytes, inflammatory exudate was processed as follows. The abscess cavity was lavaged with 0.5 ml of pH 7.4 PBS containing 1,200 U of penicillin G and 500 kallikrein-inactivating units of the protease inhibitor Trasylol (FBA Pharmaceuticals Ltd., Haywards Heath, Sussex) per ml. Procedures were performed at 4°C. Perchloric acid was added to a final concentration of 7%. The resultant suspension was centrifuged at 17,000 × *g* for 30 min. The supernatant was neutralized with 20% KOH and held at 4°C for 1 h before centrifugation at 1,500 × *g* for 10 min. This supernatant was used in the transfer experiments and is the preparation referred to as "eosinopenic factor."

RESULTS

Eosinopenic effect of passive transfer of exudate supernatant. Mice were injected intraperitoneally with 1 abscess dose of exudate supernatant after 0.45-μm filtration, and eosinophil counts were determined after 14 h. This caused a sharp fall in the numbers of circulating eosinophils (Fig. 1). By 36 h after transfer, the effect had disappeared and the eosinophil counts were similar to those of the controls.

Ultrafiltration. The material was filtered under suction through a dialysis membrane to half its original volume. Intraperitoneal transfer of the filtrate had no significant eosinopenic effect, whereas that of the residue retained the eosinopenic property (Table 1, experiment 1).

Solubility in perchloric acid. Removal of most large proteins in the exudate was accomplished by precipitation with 7% perchloric acid followed by neutralization with potassium hydroxide. Intraperitoneal transfer of the supernatant so obtained retained the eosinopenic activity (Table 1, experiment 2).

Preservation by inhibitor of proteases. During the early experiments, this substance was found to be very labile. Holding a preparation at room temperature for 1 h or at 4°C for 10 h was regularly accompanied by loss of activity. This lability could be an inherent property of the substance itself, or it could be due to the action of degradative enzymes, either endogenous or the autolytic enzymes produced by pneumococci (13). Trasylol, a protease inhibitor extracted from bovine lung, has been shown to inhibit a wide variety of proteases, including trypsin, chymotrypsin, and plasmin, and the activation of plasminogen and kallikrein (personal communication from the manufacturer). The exudate material was prepared as previously with the addition of Trasylol, (500 kallikrein-inactivating units/ml) to the buffer used in the lavage of the abscess. A similar preparation was made without the use of Trasylol. Both were held at 4°C for 24 h before intraperitoneal transfer into mice with trichinosis. The preparation wihout Trasylol had lost its eosinopenia-inducing effect; that with Trasylol retained activity (Table 1, experiment 3).

As noted above, all further experiments used

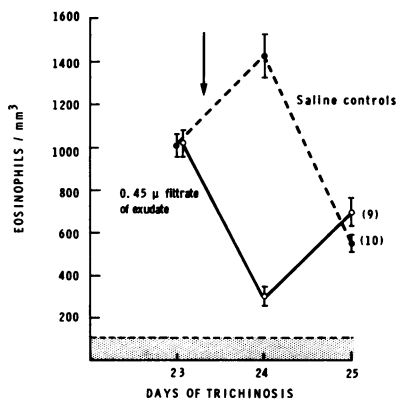

FIG. 1. *Effect of intraperitoneal transfer of filtrate of pneumococcal abscess exudate to mice during eosinophilia of trichinosis. Each recipient received the amount obtained from lavage of one abscess or a similar volume of buffered saline (at time of arrow). Eosinophil counts (mean ± standard error) were determined 12 h later. Numbers in parentheses indicate numbers of mice per point.*

TABLE 1. *Effect of procedures upon the ability of exudate supernatant to produce eosinopenia after intraperitoneal injection in mice with trichinosis*[a]

Expt	Material injected	No. of mice	Eosinophils/mm³ (mean ± standard error)		P[b]
			Before injection	14 h after injection	
1	Controls	5	540 ± 55	570 ± 50	
	Ultrafiltrate	5	510 ± 52	501 ± 54	NS
	Ultrafiltration residue	5	570 ± 32	105 ± 24	<0.01
2	Controls	5	880 ± 52	826 ± 180	
	Exudate supernatant	5	932 ± 61	160 ± 47	<0.01
	Perchloric acid supernatant	5	910 ± 58	282 ± 64	<0.01
3	Controls	6	800 ± 48	1,050 ± 146	
	EF held 24 h without Trasylol	6	804 ± 82	989 ± 120	NS
	EF held 24 h with Trasylol	6	778 ± 60	306 ± 58	<0.01
4	Controls	6	580 ± 62	1,250 ± 360	
	EF	6	571 ± 37	410 ± 97	<0.01
	EF heat inactivated at 56°C for 30 min	6	576 ± 42	927 ± 205	NS

[a] All mice received an intraperitoneal injection of 0.6 ml of experimental or control material at 7 to 9:00 p.m. on day 22 or 23 of trichinosis and had blood sampled for eosinophil counts 14 h later.
[b] Comparison of experimental and control groups. NS, Not significant.

preparation of the exudate material with addition of Trasylol, precipitation with 7% perchloric acid, and neutralization with potassium hydroxide. This material is the EF.

Heat inactivation. One-half of a preparation of EF was placed in a water bath at 56°C for 30 min. This mild heat treatment markedly suppressed the eosinopenic activity (Table 1, experiment 4).

Dose-response relationship. EF was prepared from pneumococcal abscesses of 10- and 20-h duration to provide preparations of different concentrations. Each was serially diluted and injected into mice on day 22 of trichinosis. Controls were given similarly treated PBS. Eosinophil counts were determined 15 h after transfer. The degree of eosinopenia produced was expressed as the percent suppression of the mean of control eosinophil counts. When the abscess dose of EF was compared with the percent suppression, a log dose-log response graph provided a linear regression (Fig. 2). Moreover, the lines formed by the 10- and 20-h preparations were parallel through the concentrations shown. In greater concentration there was little further suppression, and in weaker concentration suppression was undetectable. The EF obtained from an abscess of 10-h duration had approximately half the activity of that seen in the 20-h preparation.

Lack of steroid mediation. Adrenal corticosteroids may accumulate at the site of inflammation (5) and may be present in sufficient quantity to produce an eosinopenic effect on transfer. A pool of exudate material obtained

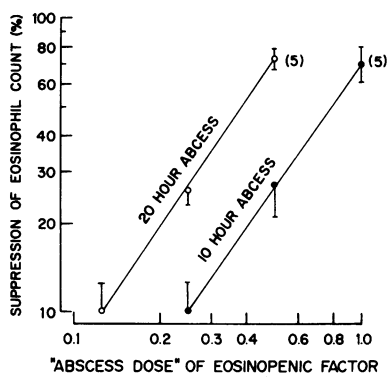

FIG. 2. *Dose-response effect of the Eosinopenic Factor preparation. One abscess dose is the amount of material obtained by lavage of one mouse pneumococcal abscess. Eosinophil counts were determined 15 h after intraperitoneal injection of EF to mice with trichinosis and expressed as the percent suppression of eosinophil counts of controls (mean ± standard error).*

from 20 abscesses was found to contain 32 ng of corticosterone per abscess as determined by the competitive protein-binding corticosterone assay described previously (1). The exudate corticosterone concentration was, therefore, 32 μg/100 ml, approximately the level of corticosterone in the serum of mice 20 h after pneumococcal infection (1). If one could assume immediate distribution (to produce the greatest possible effect) in the recipient, this would produce an increase of 1.6 μg/100 ml in the serum corticosterone concentration of the recipient mice, less

than that produced by the intraperitoneal injection itself, to which the control mice were subjected. It could not explain the eosinopenia observed.

The possibility of mediation by adrenal stimulation of recipient mice was ruled out by transfer of the EF to adrenalectomized mice. Mice were adrenalectomized on day 21 of trichinosis. Eosinophil counts were determined on day 22. The mice were divided into equivalent groups and were given an intraperitoneal injection of either EF or similarly treated PBS on the evening of day 22. Eosinophil counts were again determined 14 h later. The eosinopenic effect of the EF preparation was not prevented by prior bilateral adrenalectomy (Fig. 3).

Effects on circulating neutrophils and lymphocytes. The possible effects of EF on the numbers of circulating neutrophil leukocytes and lymphocytes were examined. Eosinophilic mice were divided into 12 groups, with at least 6 mice per group. They received 1 abscess dose of EF (or treated PBS), and blood samples were obtained after the desired intervals, in all cases between 9 and 11:00 a.m. Eosinophil, total leukocyte, and differential counts were determined. The response is a compilation of the 12 groups and required three preparations of exudate material (Fig. 4). There may have been some variation in the concentration of the exudate material, especially since that used for the 4-h determination was held for 8 h before transfer. Nevertheless, the results suggest that the eosinopenia is significant by 4 h, continues for 24 h, and is back to control levels by 36 h. There was a reduction of circulating lymphocytes by 36% of controls at 8 h after transfer. There was no significant difference between neutrophil counts of the control animals and of the EF recipients.

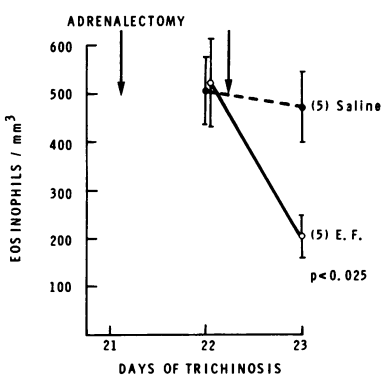

FIG. 3. *Eosinophil counts (mean ± standard error) of adrenalectomized, trichinous mice after intraperitoneal injection (second ↓) of either EF or similarly treated PBS.*

Lack of effect of killed pneumococci, pneumococcal culture filtrate, or normal mouse serum. Since the exudate was obtained from a pneumococcal abscess, possible mediation by a product of the bacteria was examined. A filtrate of a 20-h pneumococcal culture was prepared. Intraperitoneal injection of 0.6 ml, containing 0.1 ml of culture filtrate diluted as in the preparation of exudate supernatant, did not result in eosinopenia (Table 2). Pneumococci were killed by heating to 100°C for 10 min. Intraperitoneal injection of 10^7 heat-killed pneumococci also did not produce a significant reduction of the eosinophilia of trichinosis (Table 2).

Intraperitoneal injection of protein might produce a nonspecific inflammatory reaction, which in turn might cause a reduction in the eosinophil count. This possibility was examined by intraperitoneal injection of normal mouse serum. Eosinophilic mice received 0.05, 0.1, or 0.2 ml of normal mouse serum, a preparation of EF, or a similar volume of buffered saline (Table 2). Only the injection of 0.2 ml of serum

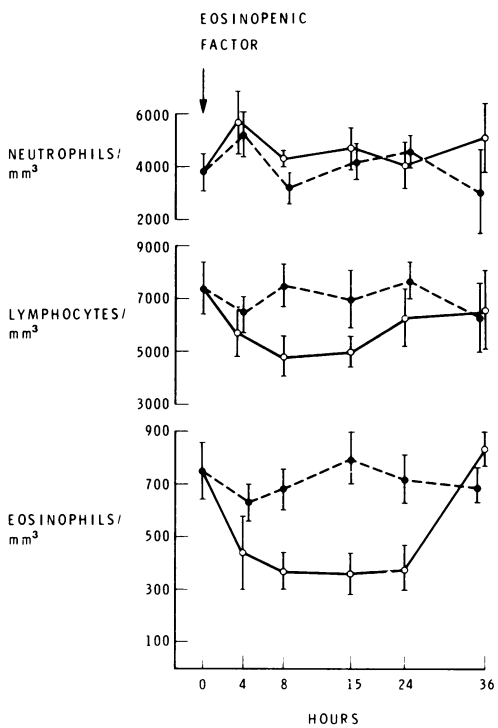

FIG. 4. *Effect of one abscess dose of eosinopenic factor (○) or similarly treated saline (●) on circulating eosinophil, lymphocyte, and neutrophil counts during the peak eosinophilic response to T. spiralis in mice. Time of intraperitoneal injection varied so that all samples were taken between 9 and 11:00 a.m. Each point (mean ± standard error) is the mean of a separate group of six to eight mice.*

produced a significant suppression of eosinophil counts, and this suppression was considerably less than that seen after injection of the EF. Preparations of EF contained an average of 0.54 mg of protein per abscess dose, equivalent to roughly 0.01 ml of serum protein. The amount of protein present in the EF was thus less than 5% of that required to produce a significant nonspecific eosinopenic effect.

Peritoneal exudate induced by EF. Lavage of the peritoneal cavity at the time of blood sampling provided an indicator of any peritoneal reaction produced by the injection of EF. Eosinophilic mice received a dose of EF intraperitoneally. Twelve hours later, blood samples were taken, the peritoneal cavity was lavaged by injection of 3 ml of PBS, and cell counts of the lavage fluid were determined (Table 3). Such a procedure can provide only a rough assessment of the total exudate; however, the lavage volume is large in relation to the small amount of spontaneous peritoneal fluid, and its content should be indicative of the relative cellular constitution of the peritoneal reaction. Little change was observed after EF administration (Table 3). The slight increase in eosinophils is probably inadequate to explain the persistent blood eosinopenia. The apparent increase in neutrophils is not statistically significant. Since the magnitude of acute inflammation sufficient to induce eosinopenia is not known, the possibility that EF might cause eosinopenia by production of acute peritoneal inflammation must be considered; however, the small increase in peritoneal neutrophils suggests that such a mechanism is unlikely.

DISCUSSION

The characteristic eosinopenia that accompanies many acute infections was first mentioned by Ehrlich in 1880 and well described by Zappert in 1893 (14). By 1914 Schwarz (10) was able to cite over 100 references confirming the occurrence of an absolute eosinopenia as a regular event during the acute phase of pneumonia, staphylococcal and streptococcal suppurative disease, erysipelas, epidemic meningitis (presumably meningococcal), typhus, typhoid, measles, varicella, rubella, cholera, and dengue. The most recent clinical review of this phenomenon was that by Weiner and Morkovin in 1952 (12). They reaffirmed the regular observation of a decrease in absolute eosinophil counts during the first several days of many acute infections, but found the degree and duration difficult to predict. They felt that the best correlation was seen with the severity of the patient's symptoms. Higher fevers were generally associated

TABLE 2. *Effect of intraperitoneal injection of pneumococcal culture filtrate, killed pneumococci, or normal serum on eosinophil counts in mice with trichinosis*

Expt	Material injected	No. of mice	Eosinophils/mm³ (mean ± standard error)		P
			Before i.p.[a] injection	14 h after injection	
1	Controls	8	871 ± 67	950 ± 56	
	Culture filtrate	6	862 ± 56	881 ± 88	NS[b]
	Killed pneumococci	6	837 ± 70	816 ± 74	NS
	EF	6	843 ± 102	81 ± 27	<0.01
2	Controls	10	981 ± 97	1,372 ± 128	
	0.05 ml of serum	5	950 ± 147	1,325 ± 122	NS
	0.1 ml of serum	5	973 ± 124	1,152 ± 98	NS
	0.2 ml of serum	5	1,080 ± 102	730 ± 117	<0.01
	EF	5	980 ± 53	270 ± 46	<0.01

[a] i.p., Intraperitoneal.
[b] NS, Not significant.

TABLE 3. *Cellular content of peritoneal lavage after EF administration*[a]

Material injected	No. of mice	Blood eosinophils/mm³		Peritoneal lavage cells/mm³		
		Before injection	14 h after injection	Eosinophils	Neutrophils	Total leukocytes
Controls	5	881 ± 53	824 ± 292	89 ± 27	752 ± 138	8,460 ± 1,057
EF	5	932 ± 61	161 ± 49	136 ± 29	1,032 ± 227	9,575 ± 628

[a] All mice received an intraperitoneal injection of Trasylol–perchloric acid-treated PBS (controls) or exudate (EF) at 9:00 p.m. on day 23 of trichinosis. Blood eosinophil counts were obtained 12 h later. Results are for peritoneum lavaged with 3 ml of PBS and cell counts obtained on resultant fluid. Results are expressed as mean ± standard error.

with more profound eosinopenia, yet the eosinophils returned to normal before subsidence of the fever in several patients. The eosinopenia did not consistently correlate with the presence of neutrophilia or with the erythrocyte sedimentation rate. Thus, although in general those patients with more symptoms, higher fevers, and more leukocytosis had greater and longer suppression of the eosinophil counts, none of these individual variables could reliably predict the eosinophil behavior in an individual patient. The eosinopenia of acute infection usually occurs even in patients with a preexistent eosinophilia; however, there have been occasional reports of instances of refractory eosinophilia, e.g., in patients with Loffler's endomyocarditis where acute infection was unable to suppress the eosinophilia (3, 9).

Eosinopenia occurs as part of the response to stress (4). This response may be duplicated by administration of glucocorticosteroids or adenocorticotrophic hormone (7). A similar eosinopenic response occurs after injection of epinephrine, although this response is also apparently dependent upon the presence of low, "permissive" levels of adrenal glucocorticoids (6). The assumption arose that the eosinopenia of acute infection represented a nonspecific manifestation of these hormonal responses to the stress of acute infection. This hypothesis was accepted by Weiner and Morkovin (12), although their series included observations of eosinopenia during localized infections such as pharyngitis, which would not be expected to exert a strong adrenal stimulation. Recent studies in eosinophilic mice demonstrated that the eosinopenic response to acute inflammation preceded the rise of serum corticosterone and occurred normally in adrenalectomized animals, without any requirement for maintenance "permissive" corticosteroid replacement (1). Thus, the eosinopenia of acute infection occurs independently of the hormonal reactions to nonspecific stress; its cause must arise in another aspect of the host response to acute inflammation. The present study demonstrates that passive transfer of material obtained from acute inflammatory exudate may duplicate such an eosinopenic response in recipient animals.

The pneumococcal infection of a subcutaneous air pouch provided a convenient source of exudate produced by a pathogen free of known toxin production. After removal of bacteria and cellular debris by 0.45-μm filtration, intraperitoneal injection of 0.1 ml of exudate into eosinophilic mice was followed by a fall in numbers of circulating eosinophils which was significant within 4 h and lasted for 24 h after transfer. The active material was retained by a dialysis membrane, was soluble in 7% perchloric acid, and was protected from destruction by autolytic enzymes by a protease inhibitor. Although these data provide only a minimal characterization of the material, they suggest that the active agent may be a large glycoprotein.

The active material is termed "eosinopenic factor" for convenience, although induction of eosinopenia may not be its only biological function. The mechanism of action of EF is not known. The rapid eosinopenic response suggests a direct effect on the eosinophil, perhaps by increasing eosinophil adhesiveness and thereby inducing intravascular margination of the cells. This effect might also occur by activation of other endogenous substances yet to be defined. Such speculations must await verification after further purification of the EF and study of its effects on eosinophil leukocytes.

The data suggest that the eosinopenic response is not merely a manifestation of an inflammatory response to the material injected intraperitoneally. Injection of 20 times the protein present in 1 abscess dose of EF produced minimal eosinopenia. Conversely, transfer of 25% of the material obtained from one abscess produced a systemic eosinopenic response of over 12-h duration in recipient mice. The material is thus active at concentrations considerably below those found in vivo at an inflammatory site.

The roles of the eosinophil, EF, and the eosinopenia of acute infection remain undefined. Further study of those phenomena distinctive to eosinophils may suggest new approaches to the role of these cells. One such phenomenon, the eosinopenia of acute infection, is duplicated by passive transfer of a material obtained from inflammatory exudate. Clarification of the character and physiological effects of this material should provide insight into one of the most distinctive aspects of eosinophil behavior.

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