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Pollen–white cell interactions

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One of the early interactions in vitro of white cells (except lymphocytes) with low ragweed pollen grains is the adherence of white cells to pollen grains. This is augmented by sera from either normal and sensitized guinea pigs or normal and atopic human subjects. Two morphologically distinct shapes of adhering white cells are evident. These consist of round and crescent-shaped white cells. Most white cells which adhere to low and giant ragweed pollen grains are round. In contrast, most white cells which adhere, in the presence of serum, to pollen grains of other species or to defatted low ragweed pollen grains are crescent shaped. Adherence is optimal under physiologic conditions, requires viable white cells, and is inhibited by glycolytic poisons. The factor or factors in the serum (pollen adherence factor[s]: PAF) which augment the adherence of white cells to pollen grains are significantly increased in sera of guinea pigs which have been previously injected with low ragweed pollen grains in Freund's complete adjuvant. PAF have been selectively removed by absorption with whole ragweed pollen grains. Antibodies to sheep red cells, skin-sensitizing antibodies, dialysis of serum, and low ragweed extracts do not significantly affect PAF activity. Heat inactivation of serum diminishes but does not stop PAF activity. The initial interactions of white cells with pollen grains in vivo, upon intraperitoneal injection of pollen, are similar to those which occur in vitro.

The antigenicity and other effects of pollen grains are not confined to the soluble products of the pollen grains. Thus, pollen grains repeatedly washed of their water-soluble products were still able to stimulate an immune response when injected into rabbits.[1] Exposure to pollen in nature occurs mostly through whole pollen grains. Many of the pollen grains are likely to land upon nasal and other respiratory mucosae, areas known to contain a variety of white cell types.[2] The interactions of white cells with whole pollen grains, as with certain

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other particulate antigens, are, therefore, likely to play a significant role in the development of immune and allergic reactions to at least some pollen antigens. Despite significant studies upon the interaction of antibodies with pollen extracts,[3] little is known about the interaction of white cells with whole pollen grains. The following study describes some of the early interactions of white cells with pollen grains which occur in vitro and in vivo.

MATERIALS AND METHODS

Pollen grains

The main part of the present study was conducted upon dried untreated low ragweed *(Ambrosia elatior)* pollen grains. A few comparative studies were conducted with pollen grains of other species and sheep red cells. The pollen grains were obtained commercially and stored up to one year at 4° C. in a dessicator. Defatted pollen grains were obtained by extraction for 24 hours in boiling ether in a Soxhlet extraction apparatus.

Pollen extracts were commercial preparations of this institute prepared by the method described by Strauss and Spain.[4] The extracts were dialyzed against physiologic salt solution to remove the phenol preservative and stored at –25° C. All the reagents were dissolved, serum was diluted, and cells were suspended in a modified Hanks solution.[5]

Absorption of serum

Whole ragweed pollen grains were suspended in serum diluted 1:5 in salt solution. The suspension was then shaken for 30 minutes at room temperature by a Thomas rotating apparatus.

Experimental animals

Most of the present studies were conducted upon randomly bred Hartley male or female guinea pigs, weighing 350 to 600 grams. Guinea pigs were treated by injection of 1.25 to 5.0 mg. of low ragweed pollen in 0.25 c.c. of Freund's complete adjuvant into each of the hind footpads. Control guinea pigs received injections of complete adjuvant only or complete adjuvant containing 30 per cent rabbit red cells. Guinea pigs treated with pollen grains without adjuvant received one intraperitoneal injection of 10 ml. of 1 mg. per milliliter of pollen in modified Hanks solution.

Prelminary experiments were conducted upon sera of 12 patients with ragweed allergy. All had symptoms of hay fever in the ragweed-pollinating season and an immediate type of skin reaction to 10 to 100 protein nitrogen units (PNU) per milliliter of ragweed extract. Nine of the 12 patients had previously received injection treatment with pollen extract. Control sera were obtained from 5 volunteers who had no history of allergies and had received no skin tests. Three sera of normal individuals who had not been out of England were obtained from Dr. Rose Augustin.

White cells

Except when mentioned otherwise, experiments were conducted upon peritoneal white cells of guinea pigs. These were obtained without preinduction of inflammatory conditions in the peritoneal cavity. Twenty to 30 ml. of modified

Hanks solution were injected into the peritoneal cavity with a syringe and a sharp No. 19 gauge hypodermic needle. The syringe was removed and the peritoneal wall kneaded several times. Peritoneal fluid was withdrawn with a syringe and a needle with a blunted point which was reinserted into the peritoneal cavity through the hole made by the intial injection of the salt solution. A total of 10 to 20 million white cells with varying numbers of red cells were usually recovered. The white cells consisted predominantly of macrophages with some eosinophils, lymphocytes, and red cells. Human and guinea pig peripheral white cells were isolated by the methods described by Lichtenstein and Osler.[6] Human nasal neutrophils were obtained from washing of nasal mucus which was removed from the nasal cavity. All white cells were washed three times with a modified Hanks solution and resuspended at a concentration of 2,500 white cells per cubic millimeter in media containing 50 per cent of modified Hanks solution and 50 per cent of media 199.

Standard reaction mixtures

Unless otherwise specified, the reaction mixtures contained the following: (1) 0.2 ml. of 2.5×10^6 per milliliter white cells; (2) 0.1 ml. of 2 mg. per milliliter of pollen grains; (3) 0.1 ml. of undiluted or diluted serum; and (4) 0.1 ml. of Hanks solution containing the reagent tested. The final volume of all the tubes was 0.5 ml. This was adjusted, when necessary, by the addition of salt solution.

Reaction mixtures were incubated at 37° C. for 60 minutes and the percentage of pollen with adhering white cells determined. This was obtained from 150 to 200 pollen grains observed at random under the phase microscope.

Sheep red cells

The components of reaction mixtures testing the adherence of white cells to red cells were similar to those of the pollen grains, except that 0.1 ml. of 0.2 per cent sheep red cells was substituted for the pollen grains.

In vivo

The guinea pigs were immobilized on their backs and left in this position for the duration of the experiment. Low ragweed pollen was introduced into the peritoneal cavity by injection of 20 ml. of 0.5 mg. per milliliter of pollen suspension. Peritoneal fluid was sampled by periodic withdrawal with a blunted needle of 0.2 to 0.5 ml. quantities. The fluid was examined under the phase microscope, and smears were made for staining with the Wright's stain. The guinea pigs were autopsied 60 minutes after pollen injection. Areas of interest were removed and fixed in 10 per cent formalin for histological observations.

Skin tests upon guinea pigs were conducted by intradermal injection of about 0.05 c.c. of 1 mg. per milliliter of pollen suspension. The sites of the injections were observed after 1, 4, 8, 24, and 48 hours.

RESULTS

In vitro reactions

Pollen adherence factor(s) (PAF). Guinea pig peritoneal macrophages and eosinophils adhered to less than 10 per cent of the pollen grains in vitro in stan-

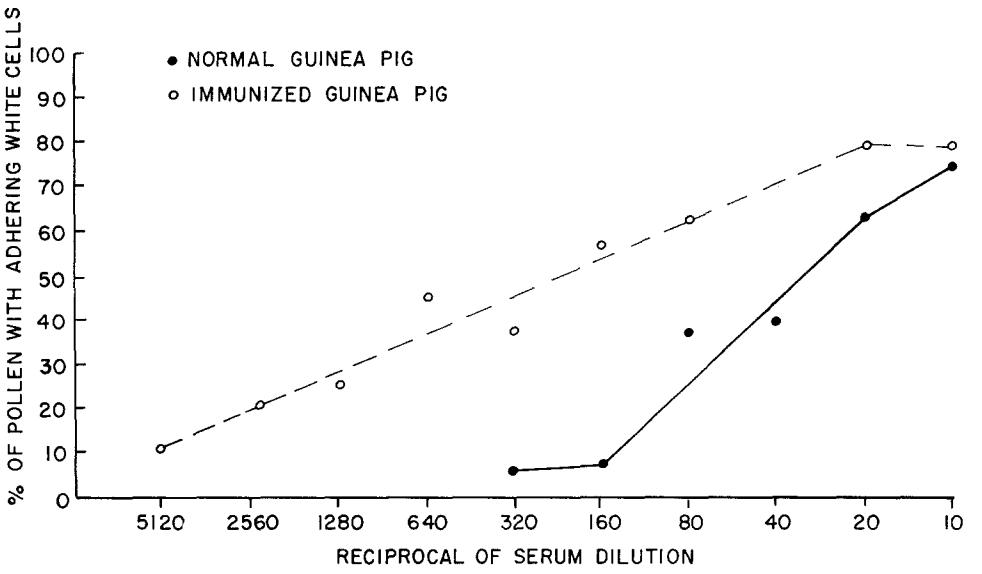

Fig. 1

Effect of serum concentration upon adherence of white cells to ragweed pollen. Pooled serum from 3 untreated guinea pigs and pooled serum from 3 guinea pigs injected 164 days previously with ragweed pollen in Freund's complete adjuvant were tested at different serum dilutions.

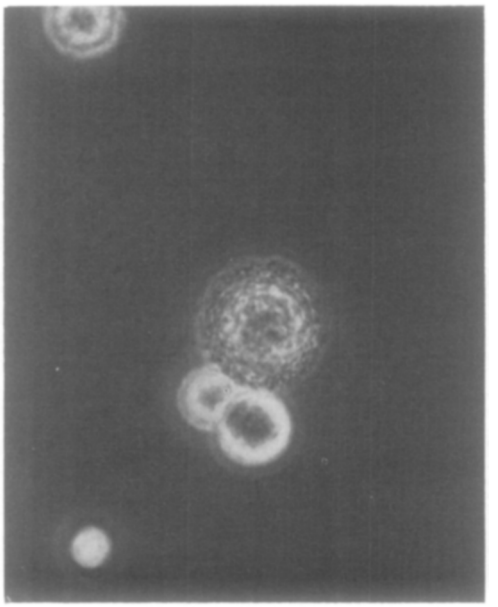

Fig. 2

Pollen–white cell aggregate photographed with a 40× phase objective and magnified originally 1,200 times. Note the round shape of the white cells which adhered to the pollen grain.

dard reaction mixtures which did not contain serum. The proportion of pollen grains with adhering white cells and the number of white cells adhering to each pollen grain were increased with the presence of serum in reaction mixtures. The increase was related to the concentration of serum (Fig. 1). It can be seen that sera from both untreated and sensitized guinea pigs augmented the adherence of white cells to the pollen grains, but serum obtained from the sensitized guinea pigs was effective at a lower concentration. The factors in the serum which augmented the adherence of white cells to the pollen grains were called "pollen adherence factor(s)" (PAF).

Morphology of adhering white cells. The adhering white cells were either round and adhered to the pollen grains with relatively small areas of their surfaces (Fig. 2) or were crescent shaped and adhered to the pollen grains with relatively large areas of their surfaces (Fig. 3). Most of the white cells adhering to the untreated low ragweed pollen were round. In contrast, most white cells which adhered to defatted low ragweed were crescent shaped.

Other cell types. Similar adherences and effects of sera were evident with human and guinea pig peripheral neutrophils, human nasal neutrophils, and guinea pig alveolar macrophages. Peripheral and peritoneal lymphocytes, platelets, and red cells did not adhere to the pollen grains either in the absence or presence of serum.

Effect of cell concentration. The percentage of pollen with adhering white cells was affected by the concentration of white cells and the concentration of pollen. There was an increase in the percentage of pollen with adhering white

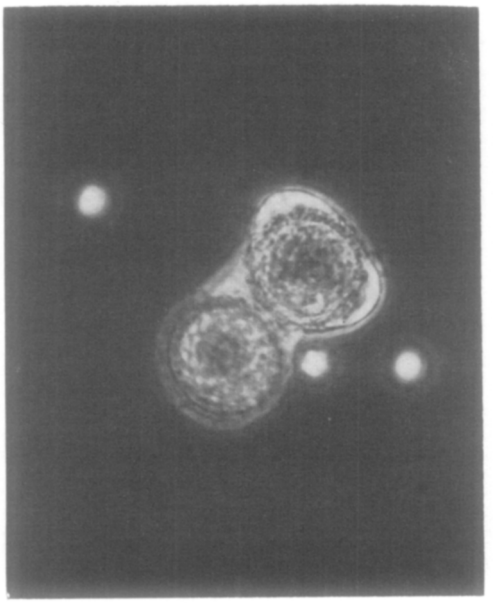

Fig. 3
Pollen–white cell aggregate photographed with a 40× phase objective and magnified originally 1,200 times. Note crescent-shaped white cells.

cells when the concentration of white cells was increased or when the concentration of pollen was decreased.

Comparative PAF activities. For comparative purposes, the reciprocal of the minimal concentration of serum which caused more than 10 per cent of the pollen grains to adhere to one or more white cells under standard conditions was arbitrarily considered as the titer of PAF. Several determinations of the PAF titer of the same serum preparation indicated that the margin of error did not exceed a factor of 2 with the same white cell preparation or a factor of 4 when different white cell preparations were used. This is similar to the maximal pre-

Table I. *Low ragweed PAF titers*

Source of sera	*Number of sera tested*	*Titers*	
		Range	*Mean*
Untreated guinea pigs	28	40- 640	193
Guinea pigs injected with low ragweed pollen in complete adjuvant	23	320-204,800	20,372
Atopic ragweed-sensitive human	12*	80- 320	213
Nonallergic humans (American)	5	80- 320	180
Nonallergic humans (English)	3	80- 320	187

*Nine of 12 were treated for variable periods with low ragweed extract.

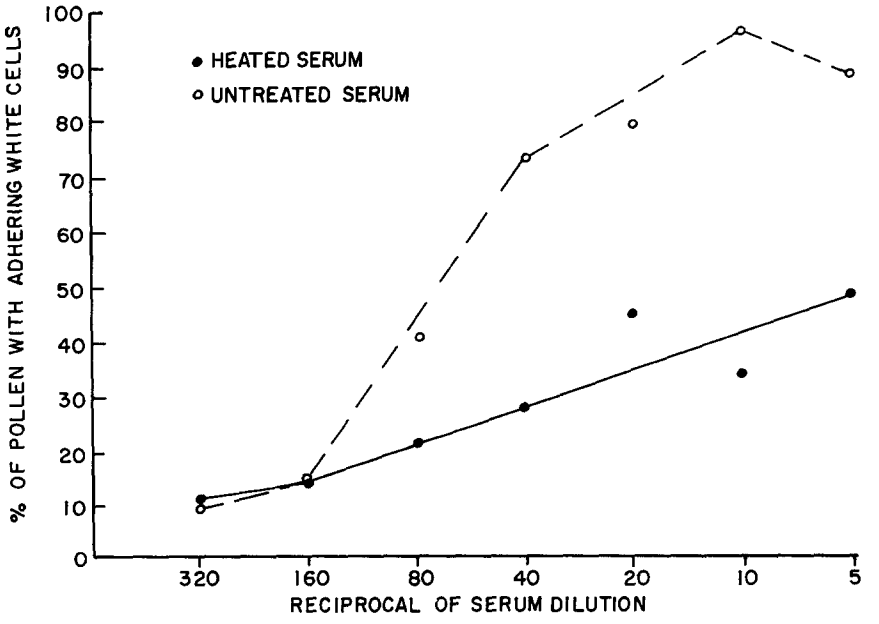

Fig. 4
Effect of heating of serum upon its PAF activity. Normal guinea pig serum, heated at 56° C. for 30 minutes, was compared with untreated serum of the same guinea pig.

cision which can be obtained during the determination of red cell agglutinin titers.[7]

Table I summarizes the results of PAF titers obtained from guinea pig and human sera. It can be seen that PAF activity was present in all sera tested. This included sera of English residents not known to be previously exposed to ragweed pollen grains. There were no significant differences between PAF titers of normal and atopic ragweed-sensitive human persons. The mean PAF titer was, however, increased over 100-fold in guinea pigs injected once with ragweed pollen in complete Freund's adjuvant. Additional titer determinations

Table II. *Effect of absorption of serum with whole low ragweed pollen grains*

*Serum**	*PAF titer†*	*Sheep red cell opsonin titer‡*
Unabsorbed	100-200	100-200
Absorbed 3 times with 4 mg./ml. of pollen grains	50-100	100-200
Absorbed 3 times with 8 mg./ml. of pollen grains	12,5-25	100-200

*Serum was obtained from control guinea pigs immunized 3 weeks previously with sheep red cells. Serum was diluted 1:5 and exposed to 56° C. for 30 minutes before absorption.

†See text.

‡Titer was the reciprocal of the lowest dilution of serum which caused over 50 per cent of the white cells to adhere to sheep red cells.

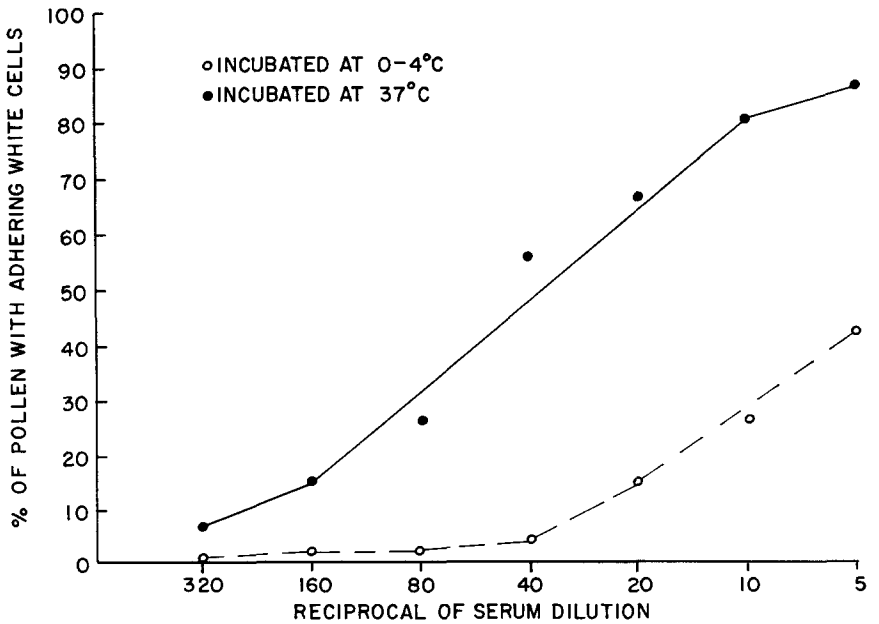

Fig. 5

Effect of incubation temperature upon adherence of white cells to pollen grains. The percentages of pollen with adhering white cells at various dilutions of normal guinea pig serum were determined after 60 minutes of incubation at 0 to 4° C. and at 37° C.

have shown that the PAF titers of sera obtained from guinea pigs injected with complete adjuvant alone or with complete adjuvant containing sheep red cells or sera from guinea pigs which were treated with pollen grains without adjuvant were not significantly different from the PAF titers of sera obtained from untreated guinea pigs.

Treatment of sera. Heating sera at 56° C. for 30 minutes diminished but did not stop the activity of the PAF. Fig. 4 illustrates typical differences between heated and nonheated sera. It can be seen that the difference between heated and untreated serum is more pronounced at relatively high serum concentrations. This is in contrast to differences between sera from normal and sensitized guinea pigs which are more pronounced at a relatively low serum concentration. Absorption of sera with whole pollen grains diminished the adherence of white cells to pollen grains but did not affect the adherence of white cells to sheep red cells (Table II). Dialysis of sera against modified Hanks solution had no detectable effects upon the PAF titers.

Effect of incubation temperature. The effect of incubation temperatures upon the adherence of white cells to pollen grains was determined in reaction mixtures of the same cell preparations incubated at 0 to 4° C. and 37° C. It can be seen in Fig. 5 that maximal adherence occurred at 37° C.

Effect of pollen extracts. The presence of pollen extract in the reaction mixtures at a concentration of up to 5,000 PNU per milliliter had little or no effect upon the PAF titers of the sera.

Effect of pH. Optimal adherence occurred at a pH range of 6.0 to 8.5. There was a reduction of 30 to 50 per cent in adherence at pH of 8.9 to 9.1. At higher pH values, there was nonspecific lysis and clumping of white cells. Adherence was mostly abolished at a pH of 5.4 or lower. White cells which were incubated for one hour in a salt solution of pH 4.6 or lower failed to adhere to pollen grains even when transferred back to a reaction mixture of pH 7.3 despite the presence of serum. Preincubation of the pollen grains at pH of 2 to 10 had no effect upon the PAF activity.

Effect of ionic composition. The adherence of white cells to pollen grains was almost completely stopped when ethylenediaminetetraacetate was added to the reaction mixtures (Table III). The PAF activity was, however, restored when either Ca^{++} or Mg^{++} was added to the reaction mixtures.

Table III. *Effect of disodium ethylenediaminetetraacetate (EDTA), calcium, and magnesium upon PAF activity*

Concentration of EDTA	*Concentration of MgCl*	*Concentration of $CaCl_2$*	*Pollen with adhering white cells (%)*
0	0	0	58
1.3×10^{-3}M	0	0	2
1.3×10^{-3}M	3.3×10^{-3}M	0	68
1.3×10^{-3}M	0	3.3×10^{-3}M	61

Dilution of normal guinea pig serum was 1:5.

Effect of metabolic poisons. Sodium fluoride and iodoacetate inhibited the adherence of white cells to pollen (Table IV), while there was little or no inhibition by potassium cyanide or sodium azide at concentrations ranging from 2.5×10^{-3}M to 1×10^{-2}M.

Other pollen grains. Preliminary studies with pollen grains of other species have shown adherences which were similar to those which occurred with low ragweed pollen grains (Table V). The following differences were, however,

Table IV. *Effect of metabolic inhibitors upon PAF activity*

Sodium fluoride concentration	*Pollen with adhering white cells (%)*	*Iodoacetate concentration*	*Pollen with adhering white cells (%)*
0	69	0	88
6×10^{-4}M	60	2.5×10^{-4}M	57
1.3×10^{-3}M	58	5.0×10^{-4}M	6
2.5×10^{-3}M	35	2.0×10^{-3}M	9
5×10^{-3}M	34	1.0×10^{-3}M	8
2×10^{-2}M	4		
1×10^{-2}M	3		

Dilution of normal guinea pig serum was 1:5.

Table V. *Adherence of white cells to various weed pollen grains*

	Without serum			*With normal guinea pig serum (1:5 concentration)*		
	Pollen with adhering white cells (%)	*Morphology of adhering white cells (%)*		*Pollen with adhering white cells (%)*	*Morphology of adhering white cells (%)*	
Pollen		*Round*	*Crescent*		*Round*	*Crescent*
Amaranthus hybridus	14	91	9	100	6	94
Ambrosia elatior (low ragweed)	10	100	0	66	65	35
Ambrosia elatior (defatted) (low ragweed)	56	76	24	91	18	82
Ambrosia trifida (giant ragweed)	9	100	0	65	63	37
Arthemesia vulgaris (common mugwort)	37	68	32	93	2	98
Chenopodium album (lambsquarter)	37	83	17	78	18	82
Chenopodium ambrosioides (Mexican tea)	31	98	2	98	26	74
Iva xanthifolia (carless weed, marsh elder)	37	100	0	95	5	95
Xanthium commune (cocklebur)	54	93	7	95	40	60

evident: (1) More cells adhered to the pollen grains in the absence of serum. (2) More of the adhering white cells in the presence of serum were of crescent shape. These differences were not evident when defatted low ragweed pollen grains were used instead of untreated pollen grains.

Sheep red cells. There was little or no adherence of white cells to sheep red cells or phagocytosis of red cells in the absence of serum or in the presence of serum obtained from untreated guinea pigs or guinea pigs treated with low ragweed pollen grains. Adherence and phagocytosis were, however, evident in the presence of serum obtained from guinea pigs previously injected with sheep red cells. Iodoacetate at concentrations which inhibited the adherence of white cells to pollen grains (Table IV) also inhibited phagocytosis of sheep red cells. This inhibition occurred in the presence of both low and high serum concentrations. In contrast to the adherence of white cells to pollen grains, the adherence of white cells to sheep red cells was not detectably affected by the inhibitor except in the presence of relatively very low concentrations of serum.

In vivo

Peritoneal cells. Upon intraperitoneal injection of low ragweed pollen, pollen–white cell aggregates were evident in about 3 to 5 minutes after pollen injection. The size of the aggregates was progressively increased up to about 20 minutes after pollen injection. This was followed by the apparent disappearance of most of the pollen grains and macrophages from the peritoneal fluid suspension. Upon autopsy, yellow clumps in the shape of nodules a few millimeters in diameter, or in the shape of thin elongated strands, were found upon various areas of the peritonium. The clumps were compact and could not be broken up even by vigorous shaking or other manipulations which did not disrupt the individual cells. Histological observations revealed that the clumps consisted mainly of white cell–pollen aggregates.

Skin reactions. Guinea pigs treated two weeks previously with low ragweed pollen in complete adjuvant usually developed erythema and swelling 8 to 24 hours after intradermal injection of pollen. Maximal reactions, often accompanied by central necrosis and blanching, occurred at 24 hours. Untreated guinea pigs or guinea pigs treated with complete adjuvant or pollen grains alone developed little or no inflammatory reactions to intradermal injection of pollen. Guinea pigs remained sensitive to intradermal injection of pollen for at least 11 months after a single injection of pollen in complete adjuvant.

DISCUSSION

Three basic morphological alternatives are evident during the incubation of white cells with pollen grains. These consist of round cell adherence, crescent-shaped adherence, and no visible morphological interaction. The crescent-shaped adherence is the result of the flow of the white cell cytoplasm around the surface of the pollen grain and thus represents advanced phagocytic processes.[8] The crescent-shaped adherence may, therefore, be considered as a modified form of phagocytosis which is probably typical of white cells which attempt to phagocytize relatively large particles. Similar crescent shapes were reported for

eosinophils during their interaction with relatively large clumps of antigen-antibody complexes.[9] The round adherence, however, represents adherence which was not followed by the more advanced phagocytic processes.

Adherences of white cells to particles have been mostly known to represent either the phenomena known as "immune adherence" or an early phase of the phagocytic phenomena. The round adherence of white cells to pollen does not seem to fall within any of these categories.

"Immune adherence," reviewed by Nelson,[10] has been defined as "the attachment of antigen-antibody-complement complexes to the surfaces of untreated primate erythrocytes or non-primates platelets." Though most immune adherence studies were conducted with erythrocytes and platelets, other "indicator particles," like connective tissue fibrils and white cells, were thought to perform similar roles in "immune adherence." "Immune adherence" is complement dependent and is independent of the activity of the indicator particles.[11] The adherence of white cells to pollen grains under the conditions reported in this study is, however, mostly complement independent and requires the active participation of viable white cells. This is clearly evident by the facts that actions which impair the viability of the white cells, such as freezing and thawing, exposure to unfavorable pH or temperature, and metabolic inhibitors, abolished the adherence of white cells to pollen grains.

Adherence which is part of phagocytosis usually occupies a relatively short period and is followed, under favorable conditions, by processes which lead to the engulfment of the particle.[12] The round-shaped adherence of the white cells was, however, not followed by more advanced phagocytic processes, despite incubation under conditions in which this occurred with pollen grains of other species.

Phagocytosis has been shown to be the result of physical factors, e.g., surface tension[13] and energy-requirng processes of the white cells.[14, 15] Similar factors are likely to affect the adherence of white cells to pollen grains. Thus, different surface tensions due to different compositions of pollen exines may determine in part the type and frequency of the adherence of white cells to pollen grains. The fact that crescent-shaped adherence was more frequent in reaction mixtures containing defatted low ragweed pollen grains than in mixtures containing untreated pollen grains indicates that round-shaped adherence was caused at least in part by lipid-associated compounds of the pollen surfaces.

An unexpected result was the effects of glycolytic inhibitors upon the round-shaped adherence to the pollen grains (Table IV) and adherence of white cells to sheep red cells at relatively low serum concentrations. While phagocytosis was generally considered to require energy expenditure by viable and active white cells,[14] adherence was thought to be a passive phenomenon since it was not affected by incubation at low temperatures.[16] This study has, however, shown that, like phagocytosis, adherence may be inhibited under certain conditions by metabolic inhibitors and low temperature.

Serum has been shown to contain several components which can augment the phagocytosis of antigenic and inert particles by white cells. These include both

heat-labile and heat-stable and specific and nonspecific factors.[12, 17-23] Several serum components may likewise play a role in the augmentation of adherence of white cells to pollen grains. Thus, the fact that heat inactivation of sera diminished the adherence of white cells to pollen grains indicates that in the untreated sera a heat-labile component or components had participated in the augmentation of the adherence of white cells to the pollen grains. The presence of specific factors in PAF is suggested by the facts that PAF activity was significantly augmented upon injection of pollen grains and was selectively absorbed by the whole pollen grains. This specificity is probably directed, mainly, to determinants associated with insoluble components of the pollen grains, since relatively large concentrations of ragweed pollen extracts had little effect upon the PAF titers.

The presence of PAF in sera obtained from untreated animals or individuals not previously exposed to ragweed pollen grains is consistent with other studies which have shown the presence of proteins absorbed to pollen grains[1] and specific agglutinins against ragweed pollen grains[24] in normal sera.

There is at present not enough information to correlate the PAF activity with other known serum ragweed activities. The PAF is probably not identical with skin-sensitizing antibody, since there seemed to be no significant differences in the PAF titers of sera from atopic and normal human beings. The PAF may, however, play an indirect role in allergic reactions which are mediated through white cell–bound antibodies[25, 26] by facilitating contact between the white cells and nondiffusible antigens of the pollen grains.

The main function of the PAF is probably the localization and eventual removal of the pollen grains and certain of their products. While the complete engulfment or encirclement of a pollen grain by one white cell may be difficult (because of the size and nature of the pollen grain), a larger number of white cells can effectively surround the pollen grain. This occurred frequently both in vitro and in vivo. The pollen aggregates formed through the action of agglutinins alone are weak and could be dispersed by slight movements of the tubes.[24] Pollen aggregates formed by the action of PAF and white cells could not be dispersed even by vigorous shaking of the tubes or by other means which did not disrupt the individual cells. The PAF and the white cells thus augment the action of the relatively inefficient agglutinins of the serum.

The factors which determine the type and frequency of adherence of white cells to pollen grains are likely to affect the fate of both the pollen grains and the white cells. This may account in part for differences in the pathogenicity of different pollen grains. This notion is consistent with studies which have related the pathogenicity of bacteria[20] and inert particles[27] to their avidity to the host white cells.

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