Failure to Transfer Bullous Pemphigoid with Serum from Patients (35421)

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(Introduced by F. C. McDuffie)

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Bullous pemphigoid is an uncommon bullous disease of the skin primarily affecting individuals 50 years of age or older. Blisters predominate in intertriginous areas, are often tense with fluid, and may measure several centimeters in diameter. Lesions form between the epidermis and dermis, in contrast to pemphigus in which they form within the epidermis as a result of acantholysis or loss of adherence of one cell to another. These histologic features permitted Lever (1) to distinguish the two diseases clearly, and the distinction became even greater when serum antibodies specific for each disease were described (2). The antibody found in pemphigoid is demonstrated readily by an indirect immunofluorescent technique which involves the use of tissue from several species of animals and is specific for certain epithelial basement membranes (3, 4); basement membranes from organs such as the kidney are not reactive. Because this IgG antibody reacts with an antigen in the basement membrane zone of affected patients, it qualifies as a true autoantibody (4, 5). In addition, using an immunofluorescent method Jordon *et al.* (6) demonstrated that this antibody binds the third component of complement. These findings are consistent with an immunologic pathogenesis for pemphigoid; however, neither the titer of serum antibodies nor the presence of complement-binding antibody has correlated well with the severity of the disease (7). Lerner and associates (8) demonstrated the pathogenetic role of antibody to glomerular basement membrane in Goodpasture's syndrome by transfusing plasma to monkeys. This encouraged us to attempt a similar experiment using high-titer plasma from patients with pemphigoid.

Methods. Monkeys. Three female rhesus monkeys, weighing from 6 to 8 lb each, were used. They were housed in metal cages and had no evidence of any disease during the course of the experiments.

Plasma. Plasma was obtained, by plasmapheresis, from three different patients with clinical, histologic, and immunofluorescent evidence of pemphigoid. The pemphigoid antibody titers, as determined by an indirect immunofluorescent test against both guinea pig and monkey esophagus (2), were 1:2560 (patient 1), 1:1280 (patient 2), and 1:10,240 (patient 3). The antibodies from patients 2 and 3 fixed complement, as determined by an immunofluorescent complement test (6), whereas the antibody from patient 1 did not.

Immunofluorescence (IF). The methods used and the specificity of the reagents have been detailed elsewhere (6). In the direct IF procedure, cryostat sections of monkey skin were stained with either fluorescein-tagged antihuman IgG or β_{1C}/β_{1A}. These conjugates had been demonstrated to react equally intensely against human and monkey antigens. In the indirect IF procedure, cryostat sections of guinea pig or monkey esophagus were overlaid with various dilutions of monkey plasma, followed by fluorescein-tagged antihuman IgG or β_{1C}/β_{1A}. The negative control for this procedure, normal plasma overlaid on the same substrates, actually acts as a control also for the *in vivo* experiments—that is, normal human serum has no affinity for epithelial basement membranes.

Method of transfusion. All monkeys were anesthetized with sodium pentobrocanal before transfusion. During each transfusion, each monkey received an amount of human

TABLE I. Data from Experiment with Monkey Transfused with Plasmas from Patients 1 and 2.

		Immunofluorescence				
			Indirect			
Day	Situation	Direct, IgG	IgG, serum diluted 1:80	IgG titer	β_{1C}/β_{1A}, serum diluted 1:20	MP[a]
0	Control	Neg	Neg	0	Neg	Neg
0	AT(1)[b]	1+	2+	1280	Neg	Not run
1		2+	1+	1280	Neg	Neg
2		2+	1+	1280	Not run	Neg
3		2+	1+	1280	Not run	Neg
4		2+	1+	640	Neg	Neg
7		1+	1+	640	Not run	Neg
7	AT(2)	2+	1+	1280	Not run	Neg
8		2+	2+	2560	2+	+
9		1+	2+	1280	1+	+
10		Neg	2+	1280	Neg	+
11		Neg	2+	—	Neg	+
14		Neg	1+	—	Not run	+
21		Neg	1+	—	Not run	Not run
21	AT(2)	Neg	3+	—	Not run	Not run
22		Neg	2+	640	1+	Not run
23		Neg	2+	640	Neg	+
24		Neg	2+	640	Neg	+
25		Neg	2+	320	Neg	+
29		Neg	1+	320	Neg	+

[a] MP = monkey precipitins to human plasma.

[b] AT(1) = after transfusion (70 ml of plasma from patient 1); AT(2) = after transfusion (75 ml of plasma from patient 2). In this experiment, plasma from two different patients was infused into the same monkey to determine if there might be differences related to individual monkeys.

plasma equal to 25 to 30% of that monkey's plasma volume. The intervals between transfusions varied from one animal to another and are detailed in Tables I, II, and III. Initially, plasma from several patients was cross-matched with monkey cells but, since no incompatibilities were found, this procedure was later discarded. It was found that human plasma could be transfused in volumes up to 30% of a monkey's plasma volume without congestive heart failure developing, if the procedure was carried out during a 3-hr period.

Biopsies. Specimens of skin were obtained with a 4-mm punch, the skin being infiltrated with lidocaine (Xylocaine) (unless the monkey was anesthetized with pentobrocanal). They were immediately frozen in liquid nitrogen and stored at −70° until sectioned. One frozen section of each specimen was stained with hematoxylin and eosin.

Collection of plasma. At the same time as specimens of skin were obtained, blood was drawn from a leg vein and mixed with heparin. The plasma was separated from the formed elements within 2 hr.

Traumatizing the skin. Since human pemphigoid lesions occur most commonly in intertriginous and other areas where trauma might contribute to their development, the skin of all monkeys was traumatized at the time of maximal fixation of antibody at the basement membrane zone. One area of skin was rubbed vigorously with the thumb every

TABLE II. Data from Experiment with Monkey Transfused with Plasma from Patient 2.

Day	Situation	Immunofluorescence: Direct, IgG	Immunofluorescence: Indirect, IgG, serum diluted 1:80	MP[a]
0	Control	Neg	0	Neg
0	AT[b]	Neg	2+	Neg
0	AF	2+	Not run	Not run
1		Neg	1+	Neg
2		Neg	1+	Neg
2	AT	Neg	2+	Neg
2	AF	1+	Not run	Not run
3		Neg	2+	Neg
4		Neg	Not run	Not run
8		Neg	Not run	Not run
10		Neg	1+	+
15			1+	+
21		Neg	±	+

[a] MP = monkey precipitins to human plasma.
[b] AT = after transfusion with 60 ml of plasma; AF = after freezing of skin with liquid nitrogen.

30 min for 2 hr; other areas were frozen, with a cotton swab dipped in liquid nitrogen, for 15 sec either with light pressure or with greater pressure sufficient to raise a blister. Other areas were irradiated with ultraviolet light from a high-pressure mercury lamp at an intensity which caused a sharp erythema. The latter procedure has been demonstrated to produce acantholytic blisters on patients with certain forms of pemphigus (9).

Results. The results of the three experiments (each monkey representing an experiment) are presented in Tables I, II, and III. By the end of the first 3-hr period of transfusion in the first experiment, antibody was bound weakly to the basement membrane, and by the next day binding had reached maximal intensity (Table I; Fig. 1). In the third experiment, maximal binding was not seen for 7 days (Table III). Fixation of antibody in the second experiment could be demonstrated only after gentle freezing of the skin and only in the area frozen (Table II).

TABLE III. Data from Experiment with Monkey Transfused with Plasma from Patient 3.

Day	Situation	Immunofluorescence: Direct, IgG	Immunofluorescence: Indirect, IgG, serum diluted 1:80	Immunofluorescence: Indirect, IgG, titer	MP[a]
0	Control	Neg	Neg	0	Neg
0	AT 55[b]	±	2+	5120	Neg
1		±	2+	5120	Neg
2		Neg	2+	2560	
2	AT 85[b]	±	2+	5120	Neg
3		1+	±	80	Neg
4		1+	2+	5120	Neg
7		2+			Neg
9		1+	2+	2560	+
18		1+	2+	5120	+
22		±	2+	2560	+
23		±			
25		Neg	2+	640	+
35		Neg	2+	320	+
46		Neg	1+	160	+
56		Neg	1+	80	+

[a] MP = monkey precipitins to human plasma.
[b] AT 55 = after transfusion of 55 ml of plasma; AT 85 = after transfusion of 85 ml of plasma.

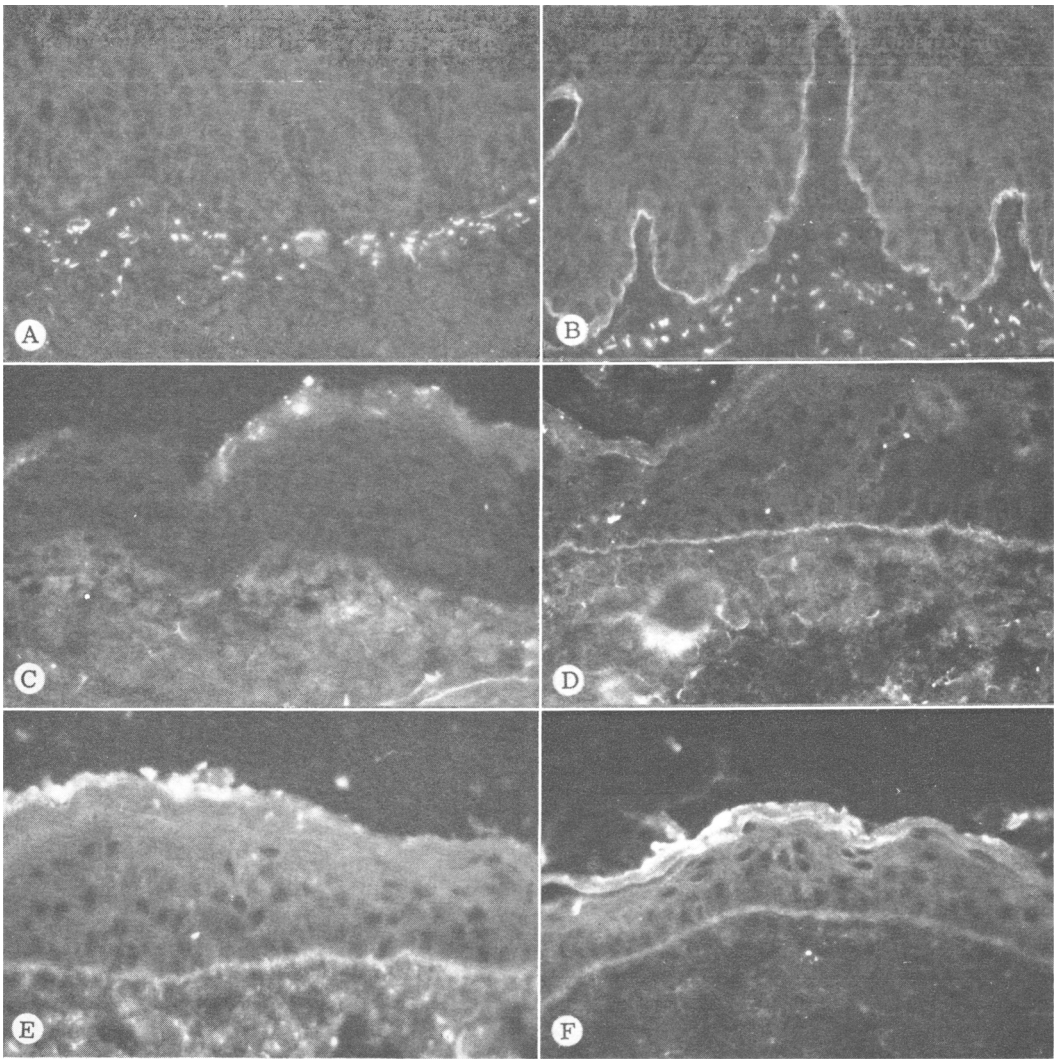

FIG. 1. Direct and indirect immunofluorescence (IF): (A) indirect IF on day 0 before transfusion; autofluorescence of elastin in upper dermis is evident. (B) indirect IF on day 1 after transfusion. (C) direct IF on day 0 before transfusion. (D) direct IF on day 0 at completion of transfusion. (E) direct IF on day 1 after transfusion. (F) direct IF on day 4.

Although antibody was bound at the same basement membrane area and with a comparable intensity to that seen in humans with active disease, no clinical or histologic evidence of lesions was found in any of these monkeys. Attempts to produce lesions by traumatizing the skin by freezing it with liquid nitrogen, by irradiating it with ultraviolet light, or by rubbing it vigorously did not produce subepidermal bullae that were not also produced in control animals by the same manipulations. Nor was there histologic evidence of blisters, either on the immunofluorescent or the hematoxylin and eosin sections. Complement was bound *in vivo* at the basement membrane zone in only the first experiment using plasma from patient 2. The same plasma was used in the second experiment (Table II) but failed to bind complement in the monkey.

In all experiments, the serum titer of antibody decreased slowly in the days after transfusion. In the second experiment, antibody failed to bind well or at all to the

basement membrane. In the third experiment, there was strong, although delayed, binding. In addition, there was a sharp decrease in titer on day 3 and an increase again on day 4. This might have been occasioned by technical problems, but the low titer was checked three times with the same result.

All three animals developed circulating antibodies, detected by immunoelectrophoresis, to the injected human plasma 8 or 9 days after the first transfusion. After this occurred in the first monkey, antibody would no longer bind to the basement membrane.

Comment. Passive transfer of large volumes of plasma from patients having bullous pemphigoid failed to produce disease in any of three monkeys. This was true even though there was strong *in vivo* binding of the antibody to the basement membrane of the skin. In addition, no blisters were produced when the skin was vigorously traumatized with an intensity short of that necessary to produce blisters in normal monkey skin.

Before concluding from these results that antibodies to basement membrane play no role in the production of skin lesions in pemphigoid, one must consider first that antibodies capable of causing skin lesions in humans may not do so in monkeys and second that prolonged exposure of basement membrane to continued high levels of antibody may be required for bullae to develop. There are no data available on the latter point, although Peck and associates (10) described one patient with pemphigus vulgaris in whom intercellular antibodies were detected 3 years before development of the cutaneous eruption. This consideration of chronicity has a counterpart in the studies of experimental glomerulonephritis, in which contact of antigen and antibody for several weeks was necessary before disease became evident (11). Another possibility is that the circulating antibody probably has less affinity for the basement membrane and may be less capable of damage compared to that already bound to the patient's skin.

Summary. Three rhesus monkeys were transfused with plasma from three different patients with pemphigoid. The antibody was bound firmly to the basement membrane zone, as determined by direct immunofluorescence, but no clinical or histologic lesions were produced. Even traumatizing the skin when antibody binding was maximal failed to produce lesions. These experiments cast doubt on the direct role of pemphigoid antibody in the pathogenesis of this disease.

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