PSEUDO B: AN ACQUIRED GROUP ANTIGEN

BY

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A number of blood samples possessing weak B antigens have been reported (Mäkelä and Mäkelä, 1955; Moullec *et al.*, 1955; Yokoyama *et al.*, 1957; Boorman and Zeitlin 1958; Levine *et al.*, 1958). Family studies on some of these have shown that the particular antigen was inherited in a recognizable way. The purpose of this report is to present evidence for the existence of an additional type of weak B antigen developing *de novo* as a temporary acquired character.

Case Report (and Materials)

Miss X, aged 64, a mild schizophrenic, was admitted to a general hospital for resection of a carcinoma of the upper part of the sigmoid colon. The diagnosis was confirmed by histological examination of the tumour.

Difficulty was experienced in determining her ABO group during routine pre-operative tests (sample 1). Her cells grouped as AB with a weakly reacting B component, while her serum contained anti-B agglutinins. The saliva contained no B substance. Blood and Saliva taken a week later showed exactly the same serological characteristics (sample 2). Between the taking of the two samples, one pint (500 ml.) of group A Rh-negative blood was transfused. Ten weeks after the collection of the second sample the patient died and a sample of blood was obtained by cardiac puncture three and a half days after death (sample 3). Miss X was not a twin. She had one brother and one sister, both of whom were tested. Her parents were dead.

Methods.—A standard tube technique was employed throughout using 2% cell suspensions. Unless otherwise stated, the incubation periods were two hours at room temperature (18° C.). Titration results were scored in the manner recommended by Race and Sanger (1958) (+ + + = 10, + + = 8, + = 5, ± = 3, w = 2, - = 0). Most of the tests were completed within two weeks of collection of the samples. Saliva and serum were stored deep-frozen and red cells were frozen in glycerol.

Results: Samples 1 and 2 Cells

As stated above, the cells gave unusual reactions within the ABO group system. Other groups were: cde/cde, MN, S+, P+(weak), Lu^a neg. Kell neg. Lewis (a-), Duffy (Fy^a). The direct anti-human-globulin test was negative.

Tests with anti-A, anti-A₁, and anti-A+B were strongly positive with very few free cells. Reactions with antiH (extract of *Ulex europaeus*) were weakly positive. Twelve anti-B testing sera at room temperature all gave positive macroscopic agglutination with many free cells. The agglutinated cells were separated by shaking with isotonic group B saliva, washed, and

resuspended in saline. Apart from their behaviour with anti-B, they were identical with the supernatant free cells. When the supernatant cells were retested with six anti-B sera at 4° C., additional weak agglutination occurred. It seemed that most of the cells contained the weak "B" type antigen, possibly in varying amounts.

The cells were agglutinated by a rabbit anti-B serum, and tests with 47 unselected human anti-B sera at 12° C. were all positive, the strength of the reactions varying considerably.

Anti-B testing serum was then diluted 1:2 with saline, and various salivas were similarly diluted and added to it. After 15 minutes at room temperature Miss X cells and B cells were added in parallel.

TABLE I.—Inhibition by Saliva of the Reaction Between Anti-B Serum and Miss X Cells

	One Volume of Anti-B Serum				
	Saliva				
	AB Secretor	A Secretor	B Secretor	O Secretor	O Non-secretor
Miss X cells	—	++	—	++	++
B cells	—	+++	—	+++	+++

As can be seen from Table I, the reaction between Miss X cells and anti-B serum was inhibited by B-containing saliva only.

Titration with Miss X cells and anti-B sera at various temperatures showed that her cells scored about half that of the control B cells and that reactions were maximal at low temperatures. High titre anti-A+B serum, completely absorbed with pooled group A cells, still reacted with B cells and Miss X cells.

Miss X cells together with A and B control cells were used to absorb a powerful anti-B serum. After each of three absorptions, the anti-B content of the supernatant serum was determined by titration against pooled B cells.

TABLE II.—Results of Absorption of Anti-B Serum with Miss X Cells and Control Cells

	Score		
	One Absorption	Two Absorptions	Three Absorptions
Anti-B after absorption with B cells	3	—	—
Anti-B after absorption with A cells	79	67	59
Anti-B after absorption with Miss X cells	72	57	49

Anti-B before absorption, score 94.

After the first absorption the sera (Table II) were examined. Absorption of the anti-B serum with B cells or Miss X cells removed the antibody for Miss X cells. Absorption with group A cells did not.

Absorption and elution tests were then made between Miss X cells and a pool of six anti-B sera. Equal volumes of cells and serum were used, and absorption at 4° C. for three hours was followed by washing four times in cold saline and elution of the antibody into saline at 56° C. The antibody dissociated more readily than with normal B cells at this temperature and a potent eluate was obtained.

From Table III it can be seen that the eluate reacts with B-containing cells only and is inhibited by B-containing saliva only.

TABLE III.—*Reactions of Eluted Antibody from Miss X Cells After Anti-B Absorption*

	A Cells	B Cells	O Cells	AB Cells
Eluate ..	—	++	—	++
Eluate + B saliva ..	—	—	—	—
„ + A „	—	++	—	++

When agglutinated cells were shaken with various salivas the agglutination could be dispersed only in the presence of B-containing saliva.

After absorption with Miss X cells, the anti-B agglutinins in the pooled sera were titrated and scored 74 (titre 1:256). The absorption was then repeated with a fresh sample of Miss X cells. An eluate prepared from these cells contained only a trace of anti-B antibody ($\pm$ reaction).

These absorption and elution tests show conclusively that Miss X cells react specifically with anti-B sera. However, the cells appear to react with only a certain fraction of the anti-B, and once this fraction has been absorbed, even though strong anti-B agglutinins remain in the serum, no further reaction with Miss X cells takes place.

The atypical B antigen appeared to be quite stable and stored satisfactorily in glycerol at -20° C. Washing the cells 10 times in saline at 37° C. did not weaken the reaction.

Miss X cells were tested with 40 group AB sera, and and 9 of these caused macroscopic agglutination. The addition of saliva of any group did not inhibit the reaction, and the antibody could be absorbed by, and eluted from, Miss X cells or "T" activated cells only. Tests at different temperatures showed that the antibody had a thermal optimum of 4° C. and was not inhibited by inactivation at 56° C. for 30 minutes. The positive sera contained no anti-A₁ or other irregular antibodies. Ficin treatment of Miss X cells increased the strength and numbers of reactions obtained. Miss X cells, therefore, in addition to possessing a weak B antigen, were polyagglutinable.

Comparative absorptions and titrations, using naturally occurring and immune anti-A antibodies, showed that Miss X cells contained a normal A₁ antigen.

Serum

Miss X serum (samples 1 and 2) contained anti-B agglutinins. Apart from a very weak cold antibody (see below), no other antibodies could be detected. Titrations against pooled B cells at different temperatures showed that the anti-B had a thermal optimum of 4° C., at which temperature the titre was 1:64 (score 51). At 37° C. it had a titre of 1:8 (score 24). No anti-B haemolysins could be detected, and the antibody could be completely neutralized by group B or AB saliva but not by group O or A. After partial neutralization with B saliva, no incomplete anti-B agglutinins could be detected by the indirect anti-human-globulin test. The serum was tested against 25 random sampled group B donors and on three occasions against pooled B cells (30 blood samples to each pool). In each instance strong positive reactions were obtained with very few free cells. In sport, the antibody gave the reactions of a normal anti-B agglutinin with no immune characteristics.

Miss X serum has been tested against her own cells in saline at 4° C., 12° C., room temperature, and 37° C., and in albumin and by indirect anti-globulin and enzyme (ficin) techniques at 37° C. At 4° C. a very weak non-specific cold agglutinin could be detected. After

absorption of this antibody with group O cells, tests between her own serum and cells were negative. As previously mentioned, the direct anti-globulin test on Miss X cells was negative.

After absorption of Miss X own anti-B agglutinin, attempts were made by inhibition techniques to demonstrate B substance in the serum. The results were inconclusive. Group A cells suspended in the serum for three hours at 37° C. failed to absorb any B substance.

Saliva

Miss X saliva was boiled in a water-bath and examined quantitatively for the presence of A, B, and H substances. A and H substances were present in about average amounts; no water-soluble B substance could be detected. Her saliva had no inhibitory effect on serial dilutions of anti-B serum (Table IV).

TABLE IV.—*Test for B Substance in Miss X Saliva*

	Group B Cells Added								
	Anti-B Dilutions								
	1	2	4	8	16	32	64	128	256
1 volume B saliva to each	$\pm$	—	—	—	—	—	—	—	—
1 volume A saliva to each	+++	+++	+++	++	+	$\pm$	$\pm$	—	—
1 volume Miss X saliva to each	+++	+++	+++	+++	++	++	+	$\pm$	—
1 volume saline to each	+++	+++	+++	+++	++	++	+	$\pm$	—

Miss X saliva did not inhibit her own anti-B antibody or the reaction between her cells and certain AB sera.

Results : Sample 3 (Post Mortem)

The third sample consisted of about 10 ml. of cells and serum obtained by cardiac puncture three and a half days after death (10 weeks after sample 2). It was in good condition and showed no haemolysis.

Cells

Differences from the previous samples were found in the ABO group. The remaining genotype was repeated and found to be unchanged. Ten anti-B grouping sera were tested separately against the cells from the post-mortem sample and cells from the original sample (stored frozen) in the parallel (Table V).

TABLE V.—*Comparison of Reaction with Anti-B Sera on Samples 1 and 3*

	Anti-B Sera Numbers									
	1	2	3	4	5	6	7	8	9	10
Miss X cells (sample 1) ..	+++	++	++	+++	+++	+	++	+	++	+++
Miss X cells (sample 3) ..	$\pm$	—	—	$\pm$	—	$\pm$	+	—	$\pm$	—

The original cell sample still gave good macroscopic agglutination, but the reactions with the post-mortem sample were almost negative. Careful microscopic examination, however, showed an occasional agglutinate of three or four cells with some of the sera. These tests were repeated with the addition of group B saliva. Some of the weak reactions were then inhibited and it was concluded that the weak agglutination was due to: (1) polyagglutination, and (2) a few surviving weak B cells. Tests with rabbit anti-B serum were negative. Absorption and elution tests showed that the cells of the post-mortem sample did not absorb the human anti-B component specific for the cells of the earlier samples.

Tests against selected AB sera showed that the post-mortem cells were still polyagglutinable, but the results were, on the whole, weaker than those obtained with the original sample.

The possibility of deterioration of group antigens in the post-mortem sample was considered. Repeated comparative titrations, however, between the cells of samples 1 and 3, using anti-A, anti-H, and anti-P (selected because of the previous weak reactions) showed the same results.

The serological evidence suggests that the group antigens in the post-mortem sample were well preserved. Previous tests on the initial sample had shown that the weak B antigen was stable. The negative reactions with anti-B serum on the post-mortem sample are therefore unlikely to be due simply to deterioration.

It is probable that the weak B antigen in the earlier samples had appeared as an acquired character. An attempt was therefore made to reproduce the phenomenon *in vitro* on the post-mortem cells by exposing them to the action of enzymes. The cells were incubated for two hours with twice the volume of a powerful bacterial "T" activating enzyme. After one wash in saline the cells were left in two volumes of a 1:6 dilution of the same enzyme at 4° C. for a further 15 hours and were then washed three times in saline. Two anti-B testing sera were pooled and absorption tests made using the above cells and untreated post-mortem cells in parallel. Absorption at 4° C. for two hours was followed by four washes in cold saline and elution into saline at 56° C. The experiment was successful and, as can be seen from Table VI, the

TABLE VI.—Reactions of Eluate from Enzyme-modified Cells and Unmodified Cells from Sample 3 After Anti-B Absorptions

Eluate from	A Cells	B Cells	AB Cells	O Cells
Untreated cells	—	—	—	—
Enzyme-modified cells	—	+++	+++	—
Enzyme-modified cells + A saliva	—	+++	+++	—
Enzyme-modified cells + B saliva	—	—	—	—

enzyme-modified cells yielded on elution a potent anti-B agglutinin. The anti-B serum that had been absorbed with the modified cells agglutinated other B cells, but failed to react with cells from samples 1 and 2.

Further tests then showed that this phenomenon was not peculiar to the cells of Miss X. Two normal group A₁ cell samples were exposed to the same enzyme under the same conditions. Absorption and elution tests, as above, showed that these cells also developed a B antigen. It was found, however, that Miss X cells from sample 1 were agglutinated by anti-B serum absorbed with the normal "pseudo B" cells, and, conversely, the eluate from these cells failed to react with Miss X cells. Clearly more than one type of "pseudo B" antigen can develop, and it is probable that Miss X post-mortem cells, already partially modified *in vivo*, developed both types of pseudo B antigen on further modification. Investigations into the reactions of these pseudo B antigens are continuing.

Serum

Anti-B agglutinins were present in the serum. The antibody had the same characteristics as in the previous samples. As before, no immune anti-B could be detected and the antibody was completely inhibited by B but not by A saliva. The serum was tested against cells from samples 1 and 2 and also cells from the post-

mortem sample at various temperatures. All the tests were negative, as also was the direct anti-human-globulin test on the post-mortem cells.

Family Study

As mentioned previously, the probandus had one brother and one sister, both of whom were tested. Her parents were dead. The brother and sister were fit and were both group A₁. Tests with six anti-B sera against their cells were negative and the cells were not polyagglutinable. The anti-B agglutinins from both their bloods reacted with the weak B antigen in the cells of Miss X.

Discussion

Miss X cells contain a weak B antigen which reacts with only a certain component of anti-B sera. Anti-titrations against her cells show that this component is most active at low temperatures and presumably naturally occurring. Weak reactions with anti-B serum on a sample of cells can be explained in a number of ways. The most obvious way is by transfusion with group AB blood. Akeroyd and O'Brien (1958) have shown that small numbers of AB cells may survive for some weeks after transfusion into group A individuals. In this case the only transfusion given was one pint (500 ml.) of group A blood after the weak B antigen had already been detected.

The large number of free cells in the original ABO group suggested the possibility of a chimera. Miss X cells, however, cannot be clearly differentiated into two types. The serological evidence suggests varying amounts of the antigen in most of her cells. Furthermore, she is not, so far as is known, a twin. A chimera was finally excluded by the disappearance of most of the B-containing cells.

van Loghem *et al.* (1957) and Stratton *et al.* (1958) have reported cases in which a presumably normal A antigen has become progressively weaker. Neither case secreted A or H substances, nor developed anti-A antibodies. The pattern is clearly quite distinct from that occurring in Miss X.

Weak B antigens with anti-B in the serum have been reported by Mäkelä and Mäkelä (1955) and by Boorman and Zeitlin (1958). B substance was present in the saliva in both instances. Moulecc *et al.* (1955) have described a family with weak cellular B antigens (B_s), but no B substance in the saliva or anti-B in the serum. Levine *et al.* (1958) have described a very weak B antigen (B_w) in the cells of a family who secrete B substance in their saliva, and Yokoyama *et al.* (1957) have described what may be a weaker variety of the same antigen (B_x).

The serological results on Miss X differ from these cases in detail, apart from the disappearance of her B-containing cells.

The experimental evidence proves that the B antigen on Miss X cells could have developed as an acquired character by the action of some agent on the group substance of her cells. Enzymic action can be shown to have taken place by the fact that her cells are polyagglutinable. Furthermore, the *in vitro* experiments, using both the post-mortem cells and normal group A₁ cells, show that under the influence of certain enzymes a weak B antigen can be made to appear. Iseki and Masaki (1953) have already shown by *in vitro* experiments that certain bacterial enzymes may modify A and O group substances and in doing so reverse their specificity. It is our opinion that *in vivo* modification of the group substance in a similar way has caused the development of the B antigen in Miss X cells.

The absence of B substance from the saliva of Miss X, together with the eventual disappearance of nearly all of the cells possessing the weak B antigen, are facts which support the hypothesis of an acquired B antigen. The family study, unfortunately, contributes very little to the investigation beyond establishing that neither the brother nor sister possesses the same antigen.

If the idea of an acquired B antigen is accepted, then the reactions of Miss X's anti-B agglutinins become interesting. It has been shown that her cells react with a specific component of anti-B sera. The absence of any reactions between her cells and her own serum suggests strongly that her serum lacks this specific component. Two possible explanations for the absence of this fraction must be considered: (1) *in vivo* absorption by her own cells, and (2) depression of her antibody-producing mechanism.

Miss X cells cannot be shown to be sensitized or coated with antibody by any technique. Furthermore, the post-mortem serum fails to react with the cells in the original sample. From this it is clear that the post-mortem serum still lacks the fraction that would agglutinate her own cells. As the post-mortem sample is almost free from weak B cells that could absorb her own antibody, it follows that auto-absorption is unlikely to be the explanation of the missing fraction. It appears most probable that her red cells have been modified and acquired a B antigen. This antigen in turn has caused a modification of her anti-B agglutinins, thus preventing a reaction with her own cells.

The chimeras that have been reported (Dunsford *et al.*, 1953; Booth *et al.*, 1957; Nicholas *et al.*, 1957) show that exposure to a foreign antigen early in foetal life can inhibit antibody production thereafter. Dunsford and Stacey (1957) have reported partial breakdown of this antibody suppression in a chimera possessing A and O red cells. Anti-A agglutinins developed in this case that were not, however, auto-agglutinating. It would appear that Miss X, whose case is here reported, confirms their supposition of immunological tolerance and establishes that foreign antigens may be tolerated, even in the presence of "homologous" antibody, providing the "foreign" antigen is present in the individual's own cells.

Summary

A new type of weakly reacting B antigen is described. B substance was absent from the saliva and anti-B agglutinins were present in the serum. Serological evidence is given to show that this antigen probably developed as an acquired character, and a technique is described by which a B antigen can be produced *in vitro* in group A₁ cells. The anti-B agglutinins in the serum did not react with the acquired weak B antigen in the cells, and possible causes for this are discussed.

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PURPURA IN KWASHIORKOR

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Purpura is a well-known though uncommon clinical finding in children suffering from kwashiorkor (malignant malnutrition, infantile pellagra), the haemorrhagic spots in these patients occurring principally over the clavicles, lower abdomen, and groin, occasionally coalescing into large purplish blotches. It is briefly referred to by some authors in the discussion of the dermatosis of malnutrition (Hanafy, 1948; Achar, 1950; Trowell *et al.*, 1954), but little work has been done to elucidate the cause of the purpuric eruption, and for the most part it appears to have been accepted as a manifestation of rupture of fragile vessels in poorly nourished tissues.

At this hospital we see from 600 to 800 cases of severe kwashiorkor annually, and most of these patients are between 6 months and 3 years old. About 80% of these children suffer from primary malnutrition, being weaned on to a diet of maize porridge and water at an early age. In the remaining 20% chronic debilitating diseases, most commonly tuberculosis and salmonellosis, are present in addition to malnutrition. Parasitic diseases such as malaria and hookworm are not endemic in this area. Mild iron deficiencies and megaloblastic anaemia with haemoglobin levels of about 8 g./100 ml. are not uncommon, but purpura is relatively rare, being seen in 3–5% of patients suffering from kwashiorkor.

It has been our impression that the purpura of malnutrition is rarely associated with obvious haematological defects, but that there appears to be an undue preponderance of tuberculosis and salmonellosis in such patients. It was with a view to elucidating this observation that we undertook the following investigation.

Materials and Methods

We investigated 23 severely malnourished children suffering from purpura who were admitted to our wards over a period of nine months. All suffered from severe malnutrition with evidence of nutritional oedema or acute nutritional dermatosis or both.

The following investigations were carried out in all cases: the bleeding-time was determined by Duke's method and the clotting-time by the method of Lee and White; haemoglobin estimation, prothrombin index (Quick one-stage method), and bile and broth cultures were performed, using venous blood; all patients were subjected to tuberculin-testing with second-strength purified protein derivative, and their chests were radiographed; rectal swabs were taken and cultured for