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BS II Lectin: A Second Hemagglutinin Isolated from *Bandeiraea Simplicifolia* Seeds with Affinity for type III Polyagglutinable Red Cells

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Abstract. BS II lectin, a second hemagglutinin isolated from *Bandeiraea simplicifolia* seeds, agglutinated the red cells from 11 patients manifesting the 'acquired B' antigen phenomenon. The results of serological studies indicate that this lectin has specificity for the type III polyagglutinable receptors rather than for the 'B-like' antigens present on 'acquired B' red cells.

Two other blood samples which did not possess 'acquired B' antigens were also found to react with BS II lectin. Both of these samples were shown to have properties similar to those reported for Tk red cells. The serological characteristics of red cells modified *in vitro* by a culture broth of *Bacteroides fragilis* suggest that type III polyagglutinable receptors are identical to those associated with Tk red cells.

Introduction

Mäkelä and Mäkelä [9] reported anti-B hemagglutinating activity in saline extracts of *Bandeiraea simplicifolia* seeds. *Hayes and Goldstein* [4] isolated from these seeds an *α-D*-galactosyl binding lectin, BS I, having a high affinity for group B red blood cells [8]. *Iyer et al.* [6] have now isolated a further lectin from *B. simplicifolia* seeds, designated BS I (G1cNAc lectin), which has a primary specificity for *N*-acetyl-*D*-glucosamine.

In serological tests BS II lectin failed to react with a large number of donor blood samples having normal ABO phenotypes, but did agglutinate the red cells from 11 'acquired B' blood samples as well as those from 2 patients who had not acquired 'B-like' antigens. *Bacteroides fragilis* was isolated by blood culture from one of these patients.

B. fragilis has recently been implicated in inducing Tk red cell polyagglutination [5]. We have studied red cells modified by this organism *in vitro*. Our results indicate that the type III polyagglutinable receptors present on 'acquired B' red cells [1] are identical to those that define Tk polyagglutination.

Materials and Methods

BS II lectin was isolated from *B. simplicifolia* seeds by affinity chromatography on chitin as previously described [6] and diluted to an initial concentration of 0.4 mg/ml with 22% bovine albumin. Bovine albumin was also used as the diluent in titration studies. Agglutination tests were performed by adding 1 vol of a 5% saline suspension of washed red cells to 2 vol of lectin. Following incubation at 4 °C for 1 h the tests were centrifuged at 1,000 *g* for 15 sec and examined macroscopically. Agglutination reactions were scored according to the method of *Race and Sanger* [10]. Sugar inhibition studies were carried out with 0.1 *M* solutions of the various sugars in phosphate-buffered saline at pH 7.3. Details of other serological techniques have been presented elsewhere [7].

Table I. Reactions of 'normal' and selected red cell phenotypes with BS II lectin

Non-reactive
O(48), A_1(52), A_2(18), B(36)
AB(43), Oh(3), A_x(5), $B^H{}_m$(1)
A_1B_m(1), Tj(a−)(3)
$P_1{}^k$(1), U neg(6), Rh_{null}(3)
Weakly reactive (titer 2, score 8)
Protease-treated (all ABO types)
Neuraminadase-treated (all ABO types)
Tn(3), Cad 2(2)
Strongly reactive (titer 32, score 47)
'Acquired B' red cells(1)

Figures in parentheses indicate the number of each cell type tested.

Results

Serological Specificity of BS II Lectin

BS II lectin did not agglutinate the red cells from 48 group O, 52 group A_1, 18 group A_2, 36 group B or 43 group AB donor blood samples. Red cells with uncommon phenotypes within the ABO, P, MNSs and rhesus blood group systems were also non-reactive. Weak reactions (titer 2, score 8) were encountered with protease or neuraminidase *(Vibrio cholerae)*-treated red cells, as well as with the polyagglutinable red cell types Tn and Cad 2. A blood sample from a patient having acquired 'B-like' antigens was found to be strongly reactive with BS II lectin. These results are summarized in table I.

10 further examples of 'acquired B' red cells were tested with BS II lectin. Although initially only 4 reacted, all did so following ficin treatment (table II). No such enhancement was observed following ficin treatment of normal, Tn, Cad 2 or neuraminidase-treated (T-activated) red cells.

An additional 236 blood samples submitted to the blood bank were tested, and the red cells from 2 patients, Mrs. L (group A_1) and Mrs. M (group B), were strongly agglutinated by BS II lectin. Although these red cells had not acquired 'B-like' antigens they were polyagglutinable. *B. fragilis* was isolated from a blood culture of Mrs. M.

Studies on B. fragilis-Modified Red Cells

Normal red cells were modified with the supernatant from the blood culture obtained from Mrs. M, and tested with BS II and *Arachis hypogoea* lectins. The results of these tests are shown in table III. The modified red cells reacted strongly with BS II lectin and weakly with *A. hypogoea*. Treatment of the modified red cells with ficin resulted in marked enhancement of agglutination by both reagents, in contrast to the

Table II. Reactions of eleven examples of 'acquired B' red cells with BS II lectin

	Case No.												
	1	2	3	4	5	6	7	8	9	10	11	A	B
Untreated													
Titer	16	0	16	0	8	32	0	0	8	0	0	0	0
Score	32	0	34	0	31	47	0	0	20	0	0	0	0
Ficinized													
Titer	512	32	512	8	256	512	8	4	128	8	16	2	2
Score	98	42	96	24	82	100	21	18	76	31	32	8	8

Table III. Results of titration studies on 'acquired B' red cells and red cells modified by *B. fragilis* with BS II and *A. hypogoea* lectins

	BS-2 lectin		*A. hypogoea*	
	untreated	ficinized	untreated	ficinized
O	0	0	0	0
A_1	0	2	0	2
B	0	2	0	2
O(BF)	64	16,000	32	256
A_1(BF)	128	32,000	64	512
B(BF)	64	16,000	32	256
Mrs. L	32	4,000	32	128
Mrs. M	16	1,000	8	64
'Acquired B'				
1	16	512	4	32
2	16	512	8	64
3	8	256	2	16
4	32	512	16	128
5	8	128	4	32
T-activated[a]	2	2	4,000	512

Tests performed at 4 °C for 1 h. BF = Cells treated with *B. fragilis* culture broth.

[a] Neuraminidase-treated.

results obtained following ficin treatment of neuraminidase-treated (T-activated) red cells.

The results of tests with BS II lectin and *A. hypogoea* on the red cells from Mrs. L and Mrs. M are also shown in table III. Ficin treatment of these cells resulted in enhancement of agglutination, similar to that observed following ficin treatment of the *B. fragilis*-modified red cells.

The red cells of Mrs. L, Mrs. M and red cells modified *in vitro* by the *B. fragilis* culture broth were shown to have properties similar to those reported for Tk red cells [5]. Agglutination in group AB serum was enhanced by ficin. There was no agglutination in *A. hypogoea* which had been absorbed with neuraminidase-treated red cells. Aggregation in Polybrene was normal. The sera of Mrs. L and Mrs. M contained normal quantities of anti-T.

Group A_1 red cells were treated with the *B. fragilis* culture broth and tested by absorption and elution techniques with group A sera and commercial anti-B reagents. No evidence for the acquisition of 'B-like' antigens could be demonstrated on these modified red cells.

Demonstration of Tk Receptor on 'Acquired B' Red Cells

Table III also shows the results of tests with *A. hypogoea* on the red cells from 5 'acquired B' blood samples. All were weakly agglutinated by *A. hypogoea,* and the reactions were enhanced by ficin. Agglutination of these 'acquired B' samples in group AB serum was also enhanced by ficin, and the red cells from each case were found to aggregate in Polybrene. Absorption of group AB serum with red cells modified by the *B. fragilis* culture broth completely removed its activity for all five samples. The sera from these 'acquired B' bloods contained anti-T.

Inhibition Studies on BS II Lectin

The extent to which various sugars inhibited the hemagglutinating activity of BS II lectin was studies. *N*-acetyl-*D*-glucosamine inhibited the agglutination of all reactive red cell samples, including those modified by *B. fragilis in vitro.*

No inhibition was observed with lactose, *D*-galactose, *D*-glucose, *N*-acetyl-*D*-mannosamine or with *N*-acetyl-*D*-galactosamine. Saliva samples (secretor and non-secretor), purified A and B substances, and hydatid cyst fluids also were non-inhibitory.

Discussion

According to *Gerbal et al.* [3] the 'acquired-B' antigen phenomenon arises from the action of a deacetylase, produced by certain strains of *Escherichia coli.* This enzyme transforms *N*-acetyl-*D*-galactosamine on group A_1 cells into *D*-galactosamine which cross-reacts with human anti-B reagents. It is evident, however, that BS II activity with 'acquired B' cells is not directly related to bacterial deacetylation for the following reasons. First, 2 patients' blood samples, one of which was group A_1, reacted strongly with BS II lectin but did not exhibit the 'acquired B' antigen phenomenon. Second, the supernatant broth from a culture of *B. fragilis* did not transform A_1 red cells into 'acquired B' red cells, although these modified red cells reacted strongly with BS II lectin. Finally, BS II lectin is inhibited by *N*-acetyl-*D*-glucosamine but not by *N*-acetyl-*D*-galactosamine or *D*-galactose,

the A and B immunodominant sugars, respectively.

An enzyme produced by certain strains of *B. fragilis* has been implicated by *Inglis et al.* [5] in causing Tk polyagglutinability. We have demonstrated that BS II lectin reacts with red cells modified *in vitro* by the supernatant from a broth culture of this organism, and *Bird* [2] found that BS II lectin reacted with *in-vivo*-induced Tk red cells. It is therefore our contention that BS II lectin recognizes the Tk polyagglutinable receptor.

The results of studies on the polyagglutinable red cells of Mrs. L, Mrs. M and the 'acquired B' blood samples indicate that these cells possess Tk receptors. Of particular significance is the observation that the agglutinins in group AB sera, which react with 'acquired B' red cells, are absorbed by red cells modified *in vitro* by *B. fragilis.*

Bird [2] also observed that BS II lectin reacts with red cells T-activated *in vivo.* This is especially significant since we were unable to demonstrate any appreciable activity of BS II lectin for red cells modified by neuraminidase *in vitro.* This suggests that certain neuraminidase-producing organisms are capable of exposing the Tk receptor *in vivo.* An alternative explanation is that more than one enzyme, each of a different bacterial origin, acts simultaneously or sequentially to modify the red cell membrane *in vivo.* Thus, in certain group A_1 individuals a bacterial deacetylase produces the 'acquired-B' antigen, while another enzyme, possibly from *B. fragilis,* exposes the Tk receptor. In those group A patients whose red cells are not susceptible to the action of bacterial deacetylase, for reasons given by *Gerbal et al.* [3], and in group O, B or AB individuals, the *B. fragilis* enzyme alone produces modification resulting in the exposure of Tk polyagglutinable receptors.

Beck et al. [1] proposed that the polyagglutination observed with 'acquired B' red cells be termed 'type III'. In view of the similarity between the polyagglutinable properties of both Tk and 'acquired B' red cells, we suggest that Tk polyagglutination be redesignated as type III.

The results of inhibition studies on BS II lectin suggest that *N*-acetyl-*D*-glucosamine is involved in the structure of the Tk receptor. The nature of the *B. fragilis* enzyme remains ill-defined and is the subject of current study.

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