Serological Behaviour of an "incomplete" and "superagglutinating" Anti-A from the Snail *Helix pomatia*

I. ISHIYAMA, A. TAKATSU, G. UHLENBRUCK, U. REIFENBERG, ST. SCHNITZLER, and O. PROKOP

Department of Legal Medicine, University of Tokyo, Hongo (Japan); Medizinische Universitätsklinik Köln-Lindenthal und Institut für Gerichtliche Medizin der Humboldt-Universität Berlin

(Z. Naturforsch. **26 b**, 171 [1971]; received November 4, 1970)

Recently, ISHIYAMA and TAKATSU [1] suceeded in converting saline agglutinating anti-A from the albumin gland of the snail *Helix pomatia* into "incomplete", non-agglutinating anti-A by reductive cleavage and subsequent alkylation according to PORTER [2]. However it is known [3], that this antibody-like anti-A is not only directed against the non-reducing terminal α-linked *N*-Acetyl-D-galactosamine (GalNAc) of blood group A substance and other glycoproteins [4], but also reacts with the terminal β-anomer of GalNAc, although the affinity is much greater to the α-glycosidically bound hexosamine [3, 4].

It was therefore of interest, to investigate the reaction of this "incomplete" anti-A with human O or B red blood cells (rbc), which had been treated with neuraminidase (RDE) and, as a consequence, had developed terminal β-linked GalNAc in their superficial glycoprotein layer, thus rendering them agglutinable by the normal snail agglutinin too. As these new uncovered receptors, which can be partly removed by proteolytic enzymes, are located in the outermost relief structure of the rbc membrane — whereas the blood group A specific antigens are exposed more in the deeper clefts or valleys of the surface architecture — we expected, that the "incomplete" snail agglutinin, while not agglutinating A erythrocytes, would still react with RDE-treated O and B rbc and also agglutinate other rbc (pigeon, duck, chicken horse) known to show superficial glycoprotein bound β-linked GalNAc after enzymatic removal of neuraminic acid.

In confirming our view [5], that the question of "incomplete" agglutinins is also a membrane problem, we found (Table I), that A rbc treated with pronase were agglutinated directly in saline by the "incomplete" anti-A, or, without treatment, were clumped by an immune serum to the heterophile snail anti-A, blocking the A receptors. In addition, A rbc "incompletely" coated with snail anti-A and subsequently incubated with blood group A substance (peptone) were not agglutinated by the immune serum to the snail agglutinin anymore, demonstrating the presence of free anti-A combining sites on the cell-bound agglutinin. On the other side, RDE- treated bird rbc were strongly agglutinated by the saline agglutinating snail agglutinin, but surprisingly and inspite of the superficial exposure of β-linked GalNAc, not by the "incomplete" form (Table I), a contrast to human O, B RDE-incubated rbc. Agglutination could however be achieved by adding soluble blood group A substance, which obviously then caused bridging of the enzymatically treated and then anti-A coated bird rbc. Another exception is represented by chicken rbc, where pronase treatment was not sufficient to reduce the thickness of the outer membrane layer, thus preventing the "incomplete" anti-A to clump the cells.

erythrocytes	normal anti-A	"incomplete" anti-A from *Helix pomatia*
human A	4.000	0
human O, B, RDE-treated	1.000	16
pigeon, chicken, RDE-treated	256	0
horse, RDE-treated	128	16
chicken, pronase-treated	4.000	0

Table I. Agglutination of different erythrocytes by normal and "incomplete" anti-A from *Helix pomatia*.

Exactly the opposite phenomenon, a so-called "superagglutinating" anti-A from Helix pomatia and Caucasotachea atrolabiata snails has been recently observed by SCHNITZLER et al. [6]: agglutinins from these special snails do not only strongly react with human A rbc (1 : 8.000 of a 10% solution), but also agglutinate O and B rbc (1 : 4), thereby obviously detecting the deeply in the membrane located glycolipid globoside I of YAMAKAWA [7] with terminal β-linked GalNAc. All these anti-A, including the "incomplete" one, were still able to precipitate with A substance and to give — with slightly differences in the pattern — precipitation lines in the immunoelectrophoresis, carried out with rabbit antisera.

It can be anticipated and deduced from our concept, that the "incomplete" reacting antigloboside I [8] will block and inhibit the above mentioned reaction with O and B rbc, and similar will do the "incomplete" anti-A from snails. It also should be possible, after reductive cleavage and interaction with other HS-containing proteins to produce artificially "superagglutinating" anti-A by inserting a foreign, elongating segment into the agglutinin without affecting its combining sites. Those investigations, as well as experiments elucidating, why not all human O and B rbc do react in the same way [6] — and how membrane-defect erythrocytes do behave with "super-agglutinating" anti-A — are in progress.

Reprints request to Prof. Dr. G. UHLENBRUCK, Abt. Immunbiologie der Mediz. Klinik, *D-5000 Köln 41*, Kerpenerstr. 15.

[1] I. ISHIYAMA and A. TAKATSU, Jap. J. exp. Med., in press.

[2] J. B. FLEISCHMANN, R. H. SAIN, and R. R. PORTER, Arch. Biochem. Biophysics, Suppl. **1**, 104 [1962].

[3] O. PROKOP, G. UHLENBRUCK, and W. KÖHLER, Vox sang. **14**, 321 [1968].

[4] G. UHLENBRUCK, G. I. PARDOE, and O. PROKOP, Forschr. Hämatol., I., p. 71, Joh. Ambros. Barth, Leipzig 1970.

[5] G. UHLENBRUCK u. O. PROKOP, Dtsch. med. Wschr. **92**, 940 [1967].

[6] ST. SCHNITZLER, G. GESERICK, W. KRÜGER, S. D. GOGOCHIA, A. B. MIRVIS u. H. A. ANNENKOW, Ärztl. Lab., im Druck.

[7] T. YAMAKAWA, S. NISHIMURA, and M. KAMIMURA, Jap. J. exp. Med. **35**, 201 [1965].

[8] J. KOSCIELAK, S. HAKOMORI, and R. W. JEANLOZ, Immunochemistry **5**, 441 [1968].