Platelet aggregation

Blood platelets are discoid cells without nuclei whose most important physiological function is apparently in haemostasis, i.e. arrest of bleeding from a wound in a blood vessel. The essential property that enables platelets to fulfil this function is their response to and activation by external stimuli—such as collagen exposed by injury to a vessel wall, ADP leaked from injured red cells or released upon activation, or thrombin formed by blood coagulation—individually, one after another, or in combination. Activation may be to varying degrees and stages depending on the nature, dose and duration of action of the stimuli. Three stages are usually recognized—shape change (from discoidal to spheroidal), aggregation and release or secretory reactions. Fully activated normal platelets undergo all stages of activation. Platelet activation has pathological overtones, contributing to atherosclerosis, metastasis or thrombosis. Aggregatory reactions of platelets may provide insights into mechanisms and modulations of intercellular interactions. But it appears now that investigating platelet activation is no simple matter and there is no straightforward answer to the question of what the order of aggregatory reactions of blood platelets is, and to several other questions about platelet activation. Traditionally platelet aggregation is regarded as being similar to colloidal particle aggregation and it is believed that the Smoluchowski theory of diffusion-controlled irreversible aggregation applies. Bikhazi and Ayyub[1] were the first to test these assumptions. They showed that these assumptions would lead to a second-order rate equation for aggregation. Their experimental data on the ADP-induced aggregation of unstirred human platelet-rich plasma, however, showed a complex time course. Second-order rate constants calculated from the data up to 45 sec ($1.4–9 \times 10^{-10}$ cm^3/platelets/sec) were 10–100 times higher than that predicted by theory. They suggested that aggregation might be surface barrier-controlled, i.e. that platelets coming close together by diffusion aggregated according to the reactivity of their surfaces. In experimental situations, however, colloidal particle aggregation rates are somewhat less than those predicted by theory, the correction factor lying somewhere between 0.5 and 0.8 (ref. 2). Furthermore, the rate constants are much higher than even the theoretical upper limit for second-order reactions in solution. This conflicts with micro-calorimetric results of Ross *et al.*[3], who found that the thermal effects of human platelet aggregation can be easily balanced (except in the case of thrombin) in the two micro-calorimeter cells, a feat well-nigh impossible for fast reactions. Interestingly Higashi *et al.*[4] found first-order kinetics for ADP-induced aggregation of human and rabbit gel-filtered platelets. They found, however, second-order kinetics for human but not rabbit platelet-rich plasma. But Nichols and Bosmann[5] found first-order kinetics. Davis and Bown[6] found a fractional order of 1.6. Hause *et al.*[7] suggested that platelet aggregation is complex and the order of the reaction might change with conditions. Jamaluddin and Krishnan[8] suggested a complex mechanism for first-order kinetics. Obviously aggregatory reactions need to be characterized carefully, employing native platelets under defined conditions, if one is to understand them.

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Galα1→3Gal-specific antibodies

Owing to the vast variation possible in their structure and conformation oligosaccharides could be much better repositories of biological information compared to proteins or nucleic acids. For example, theoretically, 3 different amino acids can form only 6 tripeptides but the same number of different hexoses can make 1056 different trisaccharides. Carbohydrate binding by glycosidases, glycosyl transferases, lectins and antibodies is crucial in any biological recognition, be it in fertilization, growth or tumour metastasis. New concepts envisage spatial and temporal variations in structure and/or concentration of glycoconjugate oligosaccharides on the one hand and carbohydrate-binding proteins on the other to modulate differentiation. Correlation of metastatic potential with certain carbohydrate sequences in some tumours holds promise for cancer therapy by biochemical engineering.

Probably due to their virtual absence in humans, α-linked galactose moieties of eukaryotic glycoconjugates, unlike their β-counterparts, had attracted much less attention. However, recently a flood of information on α-galactosides in man and other animals, vital for both the clinical immunologist and the evolutionary biochemist, has been obtained, chiefly by the work of U. Galili and colleagues, originally at the Hebrew University, Jerusalem, and presently at the University of California, San Francisco. They detected a naturally occurring antibody of the IgG class that was specific to terminal Galα1→3Gal epitopes and present in the serum of only humans and Old World monkeys[1]. Interestingly they also noted that the terminal Galα1→3Gal structure, ubiquitous in other animals, was virtually absent in man and Old World monkeys[2]. They suggested that, in humans and Old World monkeys, as production of antibody to Galα1→3Gal (termed anti-Gal) evolved, probably to counter invading micro-organisms, this epitope disappeared, by suppression of the α1→3 galactosyl transferase gene, so as to avoid autoimmune reaction.

This hypothesis evoked several questions. Anti-Gal being universal in man, how many of those unexplained autoimmune diseases could be accounted for

by the sporadic expression of the (evolutionarily) recently suppressed $\alpha 1 \rightarrow 3$ galactosyl transferase gene?—especially as J. L. Avila and colleagues from Venezuela demonstrated in 1988 a spurt in anti-Gal production accompanying parasitic infection in man[3]. Curiously the Gal$\alpha 1 \rightarrow 3$Gal-binding anti-Gal antibody leaves untouched the Gal$\alpha 1 \rightarrow 3$ (Fuc$\alpha 1 \rightarrow 2$)Gal that constitutes the B and AB blood group antigens, in what could be an example of immunological fine-tuning and tolerance[4].

Colonization of murine lung by the malignant murine cell line MO_4 depends on availability of exposed terminal α-Gal groups on these cells. Treatment with α-galactosidase therefore abolishes their lung-colonizing ability. Alternatively, covering the terminal α-Gal groups of MO_4 cells with anti-Gal from human serum could also prevent lung colonization by the cells, as shown by V. Castronovo and colleagues of the University of Liege[5]. Even the $F(ab')_2$ fragment of anti-Gal could bring about the same effect, ruling out any role for the nonspecific Fc portion of the antibody in reduction of lung colonization. In this context it may be remembered that altered glycosylation of surface glycoconjugates has been widely recognized as a hallmark of the malignant phenotype. Tumour-specific expression of $\alpha 1 \rightarrow 3$ galactosyl transferase to form α-galactoside end-groups has been noted[6]. Could anti-Gal have a scavenging role in removing such cells? Whatever be the answer, the recent insights into the role of anti-Gal and Gal$\alpha 1 \rightarrow 3$Gal epitope are bound to influence the strategy of containing infections, autoimmune diseases and tumours.

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