Evidence for the Molecular Basis of Aging and Sequestration of Mammalian Erythrocytes

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1. INTRODUCTION

This chapter represents a progress report—a story that relates the interplay of blood groups and red blood cell (RBC) survival to culminate in our current hypothesis for the molecular basis of aging and sequestration of mammalian senescent RBC from circulation. It was Ashby who, in 1919, demonstrated that RBC have a definite life span in the circulation. He accomplished this by the transfusion of RBC of blood group O into individuals of blood types A or B. He noted that, despite the compatibility of the blood transfusion, the RBC did not survive indefinitely. His data suggested an average life span of 83 days.

Since that observation, the limited life span of RBC has been demonstrated by a number of different techniques, some of which permitted a more accurate assessment of the duration of this life span—120 days in man (Berlin et al., 1959). This involves the turnover of the equivalent of $2 \times 10^{11}$ RBC/day in a normal adult (Bocci, 1981) and $1.6 \times 10^5$ of recirculations of a given RBC before its ultimate sequestration from the circulation (Allison, 1960).

The increase in density associated with increasing age of RBC provided a suitable physical method to separate RBC of different age (Piomelli et al., 1967). That made it possible to observe many time-dependent changes in RBC involving size, deformability,

Abbreviations used in this chapter: aRBC, enzymatically desialated RBC; CMP–NAN, cytidine monophosphate derivative of sialic acid; Endoglycosidase, endo-$\text{-N}$-acyetyl-$\alpha$-$\text{-d}$-galactosaminidase; FITC–LFA, fluorescein isothiocyanate-labeled LFA; FITC–PNA, fluorescein isothiocyanate-labeled PNA; FITC–WGA, fluorescein isothiocyanate-labeled WGA; Gal, $\text{d}$-galactose; GalNac, $\text{d}$-$\text{-N}$-acetyl$\text{-d}$-galactosamine; GlcNAc, $\text{d}$-$\text{-N}$-acetyl$\text{-d}$glucosamine; GOST, galactose oxidase, sialyltransferase reactive sites; IgG, immunoglobulin; LFA, Limax flavus agglutinin; NAN, N-acyetylneuraminic acid; PNA, peanut agglutinin; RBC, red blood cells; SFG(s), senescence factor glycopeptide(s); SRBC, senescent RBC, the densest fraction of RBC; WGA, wheat germ agglutinin; YRBC, young RBC, the lightest fraction of RBC.

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osmotic fragility, and enzymatic activity. These observations raised the intriguing question of whether the life span of RBC is programmed or stochastic, depending on which of the many changing parameters became the critical factor at any given set of circumstances. Extensive studies have been undertaken on the biochemical changes occurring during the life span of RBC (Fornaini, 1967; Prankerd, 1969). A recent symposium, "The Cellular and Molecular Aspects of Aging: The Red Cell as a Model," updated many of these observations (Eaton et al., 1985). The major difficulties, however, have been the unraveling of the complexities involved and the assignment of the primary signal for the sequestration of senescent RBC. In other words, which of the many changes involved also could be responsible for the ultimate sequestration of the senescent RBC?

Serendipity provided us with a plausible solution to the problem. We had long been interested in the conversion of red blood cells of A, B to the O(H) type to provide a universal blood donor type suitable for transfusion. This appeared feasible from the known chemical structures of the blood group substances (Watkins, 1972). We were successful in enzymatically converting RBC of type B to type H in rabbits, rats, and gibbons using the α-galactosidase from coffee beans and from Clostridium sporogenes (Maebashi) (Dybus and Aminoff, 1983). Autologous transfusions in rats and rabbits of the RBC treated with α-galactosidase resulted in no significant change in the life span of RBC in circulation.

We were successful also in enzymatically converting blood type A cells to O(H) (Levy and Aminoff, 1980), but the resulting transformed RBC did not survive in the corresponding autologous transfusion. This we attributed to the presence of a contaminating sialidase present in the C-perfringens extract used as the source of the α-N-acetylgalactosaminidase, as we and others had shown previously that treatment of RBC with sialidase results in their rapid sequestration from the circulation (Aminoff et al., 1976). This was typical of all mammalian but not avian species tested (Aminoff et al., 1976; Perret et al., 1978, 1981).

Asialoerythrocytes appear to be removed from circulation by the liver and spleen in the same way as senescent RBC (Aminoff et al., 1976). Removal of only 12% of total sialic acid with sialidase was sufficient for definitive sequestration to occur (Aminoff et al., 1976). These observations, taken together with the report of 10–15% less sialic acid in old as compared with young RBC (Danon, 1966; Baxter and Beeley, 1975), gave rise to an intriguing speculation: Could the natural physiological decrease in sialic acid content of RBC in circulation be a signal responsible for the removal of the old erythrocytes from circulation?

We were lured further into this controversy—and away from our initial objective of preparation of a universal blood donor RBC from type A cells—by two arguments presented to us against the acceptability of such a hypothesis: (1) the then current belief that the sequestration of senescent RBC can be attributed primarily to the rigidity of their cell membrane and their inability to negotiate the intricate capillaries successfully, especially in the spleen; and (2) that the decrease in the sialic acid content of RBC with in vivo aging could be accounted for by a loss of cell-surface carbohydrates as the membrane of RBC buds off as vesicles (Prankerd, 1969; Gattegno et al., 1976).

Our successful response to both contentions brought us greater confidence in our line of investigation and irrevocably involved us as active participants in this area of controversy. First, we demonstrated that liver and spleen macrophages can distinguish between normal RBC and those experimentally desialated with sialidase, whereas the hepatocytes could not (Aminoff et al., 1977). This emphasized the potential involvement of highly specific cell–cell interaction, i.e., that a chemical, and not necessarily a change in physical property of the aging RBC, could be involved.

Our response to the second challenge (Aminoff et al., 1981) demonstrated that over and