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Alloimmune Neonatal Neutropenia and Neutrophil-Specific Antigens

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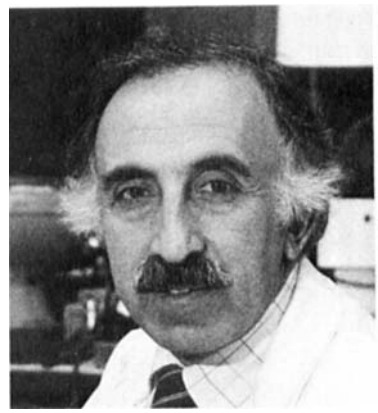

Prof. P. Lalezari

On May 12, 1946, a baby was born to DeR family in New Jersey. 6 days later the infant became febrile and died within 3 days with the diagnosis of bronchopneumonia and 'agranulocytosis'. The 2nd child developed skin abscesses 9 days after birth, and the 3rd child had both postnatal infection and a documented severe neutropenia that lasted 2½ months. The DeR family faced the same mysterious problem again in June 1958 when their 4th newborn infant developed infection and was diagnosed to have severe neutropenia. The author at that time was a medical resident at New York's Montefiore Hospital, and had initiated studies on leukocyte agglutination reaction during the previous year when a resident in hematology under Dr. *T. H. Spaet.* The case of DeR family was brought to our attention by Dr. *Murray Nussbaum,* the hematology consultant to the family, and we set out to test the hypothesis that the disorder was caused by fetal-maternal incompatibility. The potential problem in performing the experiment, however, was the finding that the parents were ABO incompatible, and the erythrocyte-leukocyte mixed agglutination reaction, observed under these test conditions, could complicate the results. Fortunately, the author was familiar with the problem. Mixed agglutination occurs when a mixture of type A or B erythrocytes are incubated with leukocytes and fresh sera containing anti-A or anti-B antibodies [*Butler,* 1956]. *Bakemeier and Swisher* [1957] had shown that participation of leukocytes in the mixed agglutination was unrelated to their ABH types and that leukocytes from type O blood donors could support the reaction. We had investigated this phenomenon further and

had found it to be composed of two separate reactions: (1) adherence of erythrocytes to leukocytes mediated by erythrocyte antibodies and (2) adherence mediated by complement. We had also observed that the stronger complement-mediated component could be inhibited by EDTA [*Lalezari,* 1959; *Lalezari and Spaet,* 1960]. Details of these observations were never published, but the information proved helpful in resolving the technical problem of leukoagglutination in the DeR family. Thus, we detected a remarkably strong leukoagglutination reaction when we added EDTA to the maternal serum. Further study revealed that EDTA had made not only the testing of ABO-incompatible cell mixture possible, but had a direct enhancing effect on the leukoagglutination reaction itself, an observation also made by *Goudsmit and van Loghem* [1958].

In preparation for our report on neonatal neutropenia [*Lalezari* et al., 1960] the author performed differential counts on numerous blood smears that had been collected from the DeR children. A marked monocytosis with a reciprocal relationship with the absolute number of neutrophils became obvious and was interpreted as a 'compensatory' phenomenon. More striking, and more difficult to explain, however, was the persistence of eosinophils and basophils. Were these granulocytes spared because they lacked the antigens expressed on neutrophils? We omitted this potentially controversial possibility from the report but continued to obtain confirming laboratory data. The first encouraging result was the finding that the agglutinated cells seen on stained slides composed only of neutrophils and did not include the lymphocytes or eosinophils. This observation could be dramatized by using granulocyte preparations from patients who had high eosinophil counts. Polyspecific leukoagglutinins from multitransfused patients, used as controls revealed an unselective reaction, producing agglutinates composed of both eosinophils and neutrophils. We became more confident when a patient with a marked leukocytosis with about 80% eosinophils became available. His neutrophils were agglutinated by the DeR plasma, but his eosinophils were neither agglutinated nor did they absorb the antibody. The transfusion-induced antibodies, by contrast, could be absorbed by these cell preparations. Additional absorption studies with erythrocytes, thrombocytes, lymph node lymphocytes, and various tissue cells established that DeR antibody was neutrophil specific, and no evidence was found to indicate the presence of the antigen on immature cells. We could now offer an explanation for the pathophysiology and the hematological features of the disorder: Antigens on neutrophils constituted a relatively small target that could be readily affected by the maternal antibodies crossing the placenta. The early myeloid cells in the bone marrow were spared because they lacked the antigens. Unfortunately, these interpretations were not readily accepted by others. Indeed, some investigators, who were working with antibodies of multiparous women, had found no adverse effect of infants' blood and, therefore, denied the existence of alloimmune neonatal neutropenia, even as a possibility. This denial entered some of the standard textbooks of hematology and casted doubts on the validity of our reports. The challenge, however, was a positive factor and stimulated the author to pursue further research. The theoretical aspects of these criticisms were easy to answer: We

proposed that maternal alloantibodies reacting with antigens with wide tissue distribution (such as HLA) could not have discernible adverse effect on fetus, because placenta may act as an antigenic barrier, and antibodies that may escape absorption by placenta would not find a confined target. A more convincing approach, however, would have been to find more examples of neonatal neutropenia and then try to define the specificities involved. During a 1959 meeting in Boston, where the study on DeR family was presented, Dr. *Leonard Luhby* informed the author of his own study on a family with 2 children affected with neonatal neutropenia. The family had been reported by *Luhby and Slobody* [1956]. Dr. *Luhby* had postulated fetal maternal incompatibility, but an antibody was not demonstrated. Similar reports also began to appear, but this time with proof for the presence of antibodies; the first one being that of *Hitzig and Gitzelmann* [1959]. The author was fortunate to receive cooperation of these investigators, and by reexamining many reported and new families, was able to define NB1 and subsequently several other antigens.

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