Leucocyte grouping : a method and its application

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Chapter five

GENERAL DISCUSSION

Although the aims formulated in chapter one have been successfully realised, it seems appropriate to discuss whether the means used were the most suitable. For instance, the defects of the leucocyte agglutination test have been discussed in extenso in chapter three. After illustrating these shortcomings, chapter three ended with the remark that the test procedures were not changed in order to preserve the homogeneity of the observations; moreover because even with these techniques a sufficient number of sera giving fairly reproducible results was available.

It might be argued that it would have been more logical first to investigate more extensively the possibilities of improving the technique.

This was not done, because it seems premature to aim at the improvement of a technique if one does not know when the test ought to give a positive and when a negative result, i.e. in this case when one is unable to recognise a leucocyte group. In chapter four is described how even with this imperfect test a leucocyte group can be recognised. In this manner the vicious circle: imperfect technique, therefore no leucocyte group, therefore no improvement of the technique, could be broken.

The essential feature of the method developed in order to recognise a leucocyte group was the use of statistical methods to overcome the shortcomings of an imperfect technique. These methods were rather time-consuming, but this was unavoidable, as can be illustrated by the following: it would have been easier to use only sera which on cross-absorption appeared to recognise only one leucocyte group. However, to be able to perform a cross-absorption experiment it is necessary to have at one's disposal donors whose leucocyte agglutination pattern with the serum under study is known. This means that one is forced to test the serum with a leucocyte panel, but it is of course of little use to perform the time-consuming cross-absorption experiment with a serum which gives a high percentage of dubious results. To be able to exclude such sera it is necessary to test the sera with a relatively large number of leucocyte samples. As the use of a large panel was thus unavoidable, the help of the computers enabled us to select with a minimum of trouble the sera which might be useful for the recognition of a leucocyte group.

A second essential, though unconventional, feature in the approach to the recognition of a leucocyte group was the use of a panel of leucocytes from women who had formed agglutinins having group Four specificity and from their husbands. When the specificity of an antibody against red cells has to be determined a panel of donors of known group-specificity is normally used. As the group-specificity of the leucocytes was unknown this was not possible. The use of the leucocytes of the couples overcame this difficulty.

These two points, combined with the circumstance that sera with leucocyte agglutinins formed during pregnancy often recognise only

one leucocyte group, were the most important factors which made the recognition of a leucocyte group possible.

Although the results obtained give an adequate answer to the question formulated in chapter one, one wonders to what extent the foregoing observations are of importance for the clinician.

The importance of these antibodies for the occurrence of non-haemolytic transfusion reactions has already been mentioned. It lies outside the scope of this discussion to relate the observations which proved the causal relationship between the presence of leucocyte agglutinins in the recipient and non-haemolytic transfusion reactions (for such a review see Walford, 74). These transfusion reactions are in general not serious, although the occurrence of fibrinolysis has been reported (9).

Brittingham has, however, shown that the injection of small quantities of serum containing leucocyte agglutinins can cause serious transfusion reactions (8). Leucocyte agglutinins formed during pregnancy can remain present in the blood up to several years after delivery. For that reason dangerous reactions can occur after the (rapid) infusion of the blood of female donors who have been pregnant (62).

As the formation of these antibodies is in so many respects reminiscent of that of the Rhesus antibodies, it is logical to look for an analogy of the morbus haemolyticus neonatorum. This affliction is indeed known as the "neutropenia of the newborn". Table XLII* shows the most important data from the cases known to me. Interesting, and unexplained, is the observation that the minimal granulocyte count is reached only 3 - 22 days post partum. The most intriguing point seems to be, that a leucopenia rarely occurs, but in most instances only a neutropenia. This could be explained as follows:

1) there exists an anti-granulocyte antibody which can pass the placenta, and an anti-lymphocyte antibody which cannot; or
2) the production of lymphocytes is sufficient to compensate for the destruction whereas the production of granulocytes is not; or
3) the leucocyte group substance is present on the granulocytes at birth but develops on the lymphocytes at a later date.

The observation that leucocyte group substance is present on the platelets and that the survival time of the platelets after transfusion is shortened in the presence of leucocyte agglutinins implies that the leucocyte agglutination reaction might be of importance in platelet transfusion practice (5). However, to be really useful the test must be both more reliable besides being complemented with techniques able to recognise thrombocyte antibodies and incomplete leucocyte antibodies and accompanied by a more complete insight into the structure of leucocyte groups. As platelet transfusions have a definite though limited field of indication in the therapy of haemorrhagic diathesis (6, 18, 61) it might be well worthwhile to pursue this line of investigation.

It is evident that leucocyte groups might also be useful in other fields such as anthropology and forensic medicine.

The possible significance of leucocyte groups for transplantation immunity has already been pointed out. Although as yet no definite information on this point is available the study of leucocyte groups forms a hopeful approach to this problem.

Leucocyte group Four fulfills the criteria of a gene-marker. As

there is no close linkage with one of the other gene-markers so far studied, the addition of leucocyte group Four can be looked upon as a real acquisition in our efforts to map the chromosome. Furthermore the data presented in chapter four indicate that the recognition of other leucocyte groups will soon be possible.

TABLE XLII

Case histories of patients suffering from neutropenia of the new-born.

Author	Previous pregnancies	Previous blood transfusions	Leucocyte agglutinins demonstrable in serum of mother	Leucocyte agglutinins demonstrable in serum of child	Lowest value of polymorphonuclear leucocytes	Lowest value of monomorphonuclear leucocytes	at day p.p.	Infection	Course	Remarks
Slobody et al. (69)	2	no	not investigated		42	4200	3	yes	recovered	
Lehndorff (42), Luhby et al. (47)	3	no	no	no	0	7800	7	no	recovered	sister of previous case
Buckwold et al. (12)	3	no information	not investigated		51	800	11	yes	died	
Hitzig (29)	0	no	yes	yes	0	8100	17[1]	yes	recovered	mother unmarried, denied abortions
Jensen (30)	3	no	yes	yes	600	1200	6[2]	yes	died	multiple congenital malformations
Lalezari et al. (41)	0	no	not investigated		135	1500	9[1]	yes	died	four cases in one family
	1	no	not investigated		not investigated			yes	recovered	
	2	no	not investigated		0	21000	12[1]	yes	recovered	
	3	no	yes	yes	0	8700	8[1]	yes	recovered	
Rossi et al. (66)	0	no information	yes	no	0	6600	6	yes	recovered	
Braun et al. (7)	6	1	yes	yes	50	8500	22	yes	recovered	

1): day of admission to hospital. Earlier laboratory data not available.

2): day of death.