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USE OF IMMUNO-FLUORESCENCE FOR THE DETECTION OF THE SENSITIZATION OF MEGAKARYOCYTES

[Following is a translation of an article by J. Duheille and H. Herbeuval in the French-language journal Comptes Rendus Soc. Biol., (Proceedings of the Society of Biologists), Vol 157, Paris, 1963, pages 150-154.]

The presence of anti-thrombocyte antibodies has been demonstrated in the case of various diseases [Note: J. Dausset, Clinical and Biological Immuno-hematology (Paris: Flammarion 1956); P. Miescher and K. O. Vorlander, Clinical and Experimental Immuno-hematology (Paris: Flammarion, 1959).], and it would be useful to find out whether this immunological process affects only the platelets or whether its action also extends to the megakaryocytes. Such a study would help us to find out whether the thrombopenias observed in these diseases are due only to the peripheral destruction of the sensitized platelets or whether the production of these platelets is upset from its very outset.

The use of immuno-fluorescent techniques has just brought us the first inklings of a solution to this problem. It is now possible, thanks to the work of Coons ([Note:] A. H. Coons, E. H. Leduc and J. M. Connolly, J. Exp. Med., 1955, Vol 102, page 49), to objectivize, with the help of a fluorescent immune serum against human globulins, the fixing on a determined cell of autoantibodies, or at least substances having antigenic properties identical to those of human serum globulins. This is the method, following McKenna and Pisciotta ([Note:] Blood, 1962, Vol 19, page 664.), that we have applied to the study of megakaryocyte platelet sensitization.

Material and Methods

We examined four patients suffering from purpuras of different origins ([Note:] We thank Mr. Neimann, who very kindly allowed us to examine the patients at the Children's Medical Clinic in Nancy), which could be divided into two acute infantile purpuras, of which one showed serous anti-platelet antibodies, one purpura accompanying a pancytopenia, and one non-thrombopenic purpura.

Medullary fluid obtained by sternal puncture from each patient was spread in thin smears on microscopic slides, either immediately or else after collection on a non-formaldehyde preserving fluid and triple washing, as in our technique for the study of leucocyte sensitization ([Note:] J. Duheille, H. Herbeuval, F. Bellut and Y. Badonnel, C. R. Soc. Biol., 1962, Vol 156, page 2093).

The dried smears were fixed for one minute in a bath of ethyl alcohol at a temperature of 95 degrees, then rehydrated in three baths of packed physiological serum (ClNa 0.14 M., phosphates of Na 0.01 M, pH = 7.2), over a period of fifteen minutes.

They were then covered with fluorescent serum against human globulins (immune serum from a goat, joined to fluorescein isothiocyanate, after a modified Riggs technique [see last note] and adsorbed on powdered organs immediately before use). The antigenic-antibody reaction is allowed to continue in a damp enclosure at laboratory temperature over a period of thirty minutes, then the preparations are cleansed of any excess of fluorescent antiserum by four baths of packed physiological solution, each lasting five minutes. This length of bath represents the minimum, and in one case lengthening it to twenty-four hours noticeably improved the contrast of the preparations by eliminating a non-specific fluorescence due to traces of unlinked fluorescein.

The preparations were mounted in packed physiological serum under sealed glass covers, and examined under the microscope both in ultraviolet light and in phase contrast (Wild apparatus). They lasted hardly twenty-four hours and therefore it is valuable to record photographically the results obtained.

Results

1. Reading the preparations. On the smears of unwashed marrow the interstics separating the cells were filled with dried plasma, fixing the marked immune serum and brilliantly outlining the cell contours. The preliminary washing of the marrow suppressed this phenomenon and the background

of the preparation appeared uniformly dark. In both cases most of the elements that appeared did not fix the marked immune serum, and were distinguished only by a weak auto-fluorescence. Some cells stood out on this dark background, identifiable by phase contrast either as plasmocytes or as eosinophilous granulocytes since the fluorescent serum still contained, in spite of adsorption on powdered organs, traces of unlinked fluorescein.

When the megakaryocytes are sensitized the fluorescent immune serum attaches itself to the cytoplasm, to which it gives an intense luminosity, clearly differentiated from the scarcely observable luminosity of the other medullary elements. The nucleus, on the other hand, is only slightly fluorescent and its outline shows up in negative on the brilliant cytoplasmic background (figure 1). It is easy to identify these cells by passing from ultroviolet lighting to phase contrast: The important dimensions, the granular appearance of the cytoplasm, the multilobed contours of the nucleus, all signal the fact that they belong to the megakaryocyte series (figure 2). The youngest units in the megakaryocyte mass, corresponding to little promegakaryocyte or to megakaryoblasts, usually escape sensitization as far as one can determine from figures 1 and 2.

In negative preparations, as in the marrow of healthy subjects, the magakaryocytes show only a weak fluorescence, scarcely more distinct than that of the other cells and not to be compared with that of the sensitized megakaryocytes. It is possible that an improvement in the immunological specificity of the marked anti-serum would get rid of this slight residual fluorescence.

2. Clinical correlations. Out of the four medullary samples we examined, two (coming from two cases of acute infantile thrombopenic purpura) presented a very clear megakaryocyte sensitization. One of these patients, with a severe attack, had received many blood transfusions in the three months preceding the examination; the presence of immune anti-megakaryocyto-platelet antibodies cannot, therefore, be ruled out. But such antibodies, likely to sensitize heterologous megakaryocytes, would on the other hand (according to all probability) have no effect on those of the patient himself. We are therefore led to think that the observed sensitization is really the work of auto-antibodies. The second patient, whose serum contained anti-platelet antibodies discernable by ordinary techniques, had also developed antibodies directed against his own megakaryocyte line.

The two negative samples corresponded one to a purpura secondary to a pancytopenia and the other to a purpura where no examination could reveal the participation of the megakaryocyto-platelet line.

Discussion and Conclusion

Our results differ on several points from those published by McKenna and Pisciotta: while these authors observed megakaryocyte sensitization only in cases of chronic idiopathic thrombopenic purpura, our two positive cases involved children suffering from acute thrombopenic purpura. It is possible that, in these two cases, it was a matter of an initial episode announcing the onset of a chronic purpura, although the isolated nature of this attack and its development to total recovery hardly plead in vaor of such a hypothesis. It is also possible that the different nature of the serum against human globulins that was used was responsible, in part, for this disparity: Our anti-serum came from hyperimmunized goats while that of McKenna and Pisciotta was a horse immune serum. A rabbit immune serum gave these authors no results. It could be that the goat immune serum usually shows up a globulin other than the 2-globulin revealed by the horse immune serum and characteristic of chronic idiopathic thrombopenic purpura. The nature of the globulin shown up by our study therefore merits further study. We can assume, however, that fibrin is not concerned since the positiveness of the reaction persists even after collection of the marrow on anticoagulant and multiple washings.

All the same, it is not possible, given these results only, to attribute a pathogenic role to the anti-megakaryocyte auto-antibodies thus revealed: they could be only the secondary signs of underlying processes, for, in one of our cases, we have seen them persist in spite of the complete and perfect clinical recovery of the patient: they could in this case have the value only of an immunological scar.

Although the limited number of cases studied in this preliminary work does not yet allow us to appreciate fully the value of this new method of detecting magakaryocyte sensitizations, the first results obtained let us nevertheless see numerous possibilities offered by this technique in the field of immuno-hematology ([Note:] Work carried out with the help of the National Institute of Health and the National Social Security Fund).

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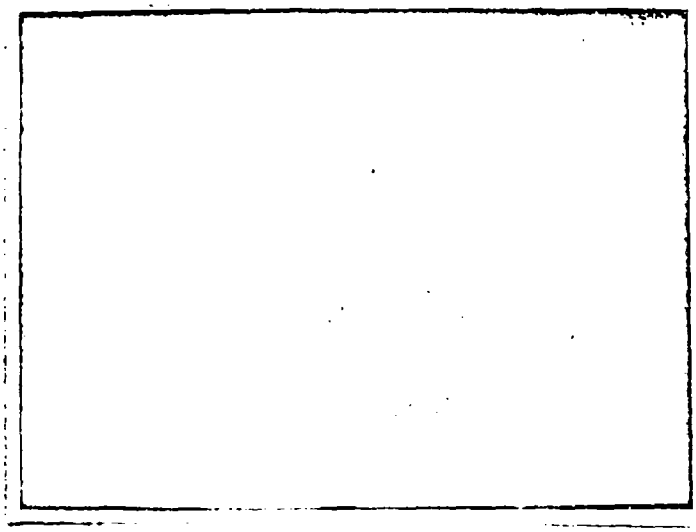

GRAPHIC NOT REPRODUCIBLE

Figure 1. Thrombopenic purpura. Sternal bone marrow, unwashed, colored by a fluorescent immune serum against human globulins. In the center, two sensitized megkaryocytes, strongly fluorescent, and a nonfluorescent megakaryoblast. Plasmatic fluorescence in the interstices separating the other cells.

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Figure 2. The same field in phase contrast.

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