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OBSERVATIONS ON THE ANTI-HUMAN-GLOBULIN TEST IN MALIGNANT DISEASE

BY

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It has been known for some time that positive anti-human-globulin reactions do occur in certain cases of malignant disease (Hyman, 1954; Rosenthal, Pisciotto, Komminos, Goldenberg, and Dameshek, 1955), and no claim to disprove this is being made. However, it was found that the high percentage of positive reactions recorded by Green, Wakefield, and Littlewood (1957) was at variance with our experience here, and this stimulated a survey of a series of cases of various types of cancer at this hospital.

Methods

1. *Direct Anti-globulin Test*.—This was performed by the method described by Dunsford and Bowley (1955). A 50% suspension of four-times-washed red cells was made and one drop of this added to one drop of anti-human-globulin serum on an opal glass tile, which was rocked gently and read after seven minutes. For these tests the following anti-globulin sera were used: (1) standard rabbit anti-human-globulin serum, as supplied by the M.R.C. Blood Group Reference Laboratory; (2) rabbit anti-human-globulin serum, as supplied by Burroughs Wellcome Ltd.; (3) hyper-immune horse serum, as supplied by the Pasteur Institute, Paris; and (4) goat anti-human-globulin serum, as supplied by Dr. J. S. Campbell, Aberdeen and North-east Scotland Blood Transfusion Service. All the tests were carried out at four dilutions from 1:10 to 1:80 in the hope that the lower dilutions might demonstrate an antibody not evident at the dilution optimal for rhesus work. The sera 1, 3, and 4 contained anti-non-gamma-globulin fractions.

2. *Indirect Anti-globulin Test*.—This again was performed in the manner described by Dunsford and Bowley (1955). Two drops of 50% suspension of washed group O rhesus-positive cells were added to four drops of patient's serum under test, the mixture was incubated for two hours at 4° C. and 37° C., following which the suspensions were washed four times in normal saline at room temperature and one drop of the washed suspension was added to one drop of anti-human-globulin serum on an opal tile, rocked gently, and read after seven minutes. The anti-globulin serum was used in dilutions of 1:10 to 1:80 as in the direct test.

Controls.—(a) Group O rhesus-positive cells sensitized with a weak incomplete anti-D serum. (b) The red cells under test + saline.

Results

1. *Direct Anti-globulin Test on Fresh Red-cell Samples*.—A series of 100 cases of malignant disease, taken at random, was tested. These included tumours of breast 35, the alimentary system 21, the genito-urinary system 15, and the respiratory system 6, reticulosos 10, and generalized carcinomatosis 3. Ten others, including tumours of the skin and brain, and two cases of sarcoma, were investigated. Of the cases investigated, 20% had haemoglobins below 8.7 g. per 100 ml. at the time of testing. Approximately 45% of the cases tested were known to have secondary involvement. As stated above, the series was taken at random, and consequently tests were performed in some instances prior to treatment and in others after surgical intervention or during a course of radiotherapy. In none of the above-mentioned cases was a positive direct anti-human-globulin test encountered.

2. *Direct Anti-globulin Test on Stored Red Cells*.—In six cases where the clotted blood samples had been stored in the laboratory refrigerator overnight at 4° C., positive direct tests were elicited at all dilutions of anti-globulin serum from 1:10 to 1:80. In these cases identical tests were repeated on fresh samples of blood and were found on this occasion to be negative. It was concluded that during their storage at 4° C. the cells had become coated with an incomplete cold auto-antibody (Dacie, 1950) and this had been responsible for the positive results.

Indirect Anti-globulin Test.—The sera of 40 cases giving negative direct tests were incubated with normal fresh group O rhesus-positive cells as described above, for two hours at both 4° C. and 37° C. Sensitization was achieved at 4° C. in all cases except one. The reactions were stronger at the lower dilutions of the anti-globulin serum, an observation also noted by Dacie (1950). The 40 sera were also absorbed with secretor saliva to remove the incomplete cold auto-antibody and then tested against the group O rhesus-positive cells in the same way as the unabsorbed sera. All tests were negative at both 4° C. and 37° C. except in one case. The one exception to this gave a strongly positive indirect test at both temperatures in the pre- and post-absorption samples, and the antibody was therefore apparently not related to the incomplete cold type demonstrated in the other sera. It was subsequently found, however, that this serum was from a patient of group B rhesus-negative, who had received three bottles of rhesus-positive blood six months previously, and that the serum now contained incomplete anti-D and anti-C.

Discussion and Conclusions

It was not possible to confirm the high percentage of positive results obtained by Green *et al.* (1957). Antisera were used which were known to contain "anti-non-gamma-globulin" fractions, and as all the tests were performed with the anti-globulin serum in dilutions 1:10 to 1:80, it is unlikely that an antibody would have resisted detection. A possible explanation for the discrepancy is the fact that if blood samples are stored for a period at 4° C. the cells become coated with an incomplete cold auto-antibody known to be present in all normal human sera (Dacie, 1950). That this was a possible cause was shown by the fact that samples giving a negative result when fresh became positive if allowed to stand overnight at 4° C.

An interesting case encountered during this series was that of a patient with carcinomatosis showing a haemolytic process, in whom the red-cell survival was found to be diminished (a conclusion reached by using red cells tagged with ⁵¹Cr): anti-globulin tests were performed on two occasions, and there was no evidence serologically for the existence of antibodies. The bone marrow in this patient was found to be megaloblastic.

Summary

Direct anti-globulin tests on the red cells from fresh clotted blood from 100 cases of malignant disease gave negative results. Positive results were obtained if the specimens were stored overnight at 4° C.

Indirect anti-globulin tests on the sera from some of these cases gave positive results against fresh group O rhesus-positive cells at 4° C. only, but this was abolished by previous absorption of the serum with secretor saliva. It was considered that the antibody involved was the incomplete cold auto-antibody described by Dacie (1950). Possible reasons for the discrepancy with other workers are discussed.

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Medical Memorandum

Caeco-colic Intussusception Following Appendicectomy

In 1953 Cleland found records of only five cases of intussusception in the immediate post-operative period following appendicectomy, and to these he added a personal case. A further case is here recorded.

CASE REPORT

A boy aged 10 was admitted to hospital on December 8, 1956, with a history of abdominal pain for 18 hours and the signs of acute appendicitis. Appendicectomy was performed the same evening through a muscle-splitting incision, and an acutely inflamed appendix, wrapped in omentum, was found, together with some free turbid fluid. The appendicectomy was carried out with the customary ligation of appendix stump and purse-string invagination, without carbolizing the base, and the abdomen was closed without drainage.

Recovery was at first uneventful, and an enema was given on the fourth post-operative day with a good result, but on the fifth evening he was seen because of a sudden attack of severe colicky pain, which made him cry out and draw up his legs in each spasm. On examination the general condition was good, pulse and temperature were normal, and the abdomen was soft between spasms, with no distension. Palpation over the ascending colon seemed to induce painful spasms and guarding, but no mass could be felt. Bowel sounds were slightly increased. Rectal examination was negative. Two hours later he vomited, but was easier, and soon went to sleep.

At 7 a.m. the next day he had a recurrence of the severe pain, and he vomited again, though his general condition was unchanged and pulse and temperature were still normal. However, inspection of the abdomen showed fullness on the right side, while palpation suggested a vague mass in the upper right abdomen, and induced severe pain and spasm. Bowel sounds were increased. The lower rectum contained constipated faeces, and inspection of the finger-stall revealed a small fleck of blood-stained mucus, more evident when wiped on to a piece of lint. A clinical diagnosis of intussusception was made. A portable scout x-ray examination

of the abdomen was made, but it added no further information. A white-cell count was 13,500 per c.mm.

At operation 14 hours after the onset of symptoms, through a right paramedian incision, a few ounces of clear yellow free fluid was found and the diagnosis of intussusception confirmed, the apex having reached the middle of the transverse colon. Reduction was at first easy, but towards the end the ensheathing layer serosa split, and required oversewing. The whole was quite viable and little altered in colour, except for the apex. The small bowel was noted to be only slightly distended. The apex was the previously inverted base of the appendix, and in the final reduction a little pus exuded from the centre of the purse-string, while the caecum for 2 cm. round was firm with oedema and reddened. The pus was let out and mopped away, and a little penicillin-sulphathiazole powder applied. No attempt was made to fix the caecum to the parietes, and the abdomen was closed without drainage.

Post-operatively, intravenous N/5 glucose-saline was given, 1 pint (570 ml.) six-hourly for 48 hours, and fluids only by mouth were continued to four days, when solids were started. Tetracycline, 250 mg. six-hourly, was given with the intravenous fluid, then changed to 125 mg. six-hourly by mouth for three further days, in view of the abscess found at the appendix stump. On the third day the child developed a right lower lobe collapse, which readily cleared with intensive physiotherapy. Three weeks after the second operation he returned home. He has since been seen twice at the out-patient department and has been well, with a good appetite, regular bowel action, and no chest complaint; both wounds were soundly healed.

COMMENT

In the seven recorded cases, including the present one, the age of the patient has been between 4 and 28 years. With one exception (on the twelfth day), the onset has been remarkably constant at about the fifth post-operative day.

The difficulty in diagnosis has been stressed by previous writers. Slight post-operative colic is not unusual some days after abdominal operations, but the severity of the intussusception colic was striking. Abdominal palpation is rendered difficult by the recent tender wound, though a mass was felt in four of the seven cases. Cleland stated that the absence of haemorrhage from the bowel seemed absolutely constant, but this is refuted by the present case.

In four recorded cases and in the above one a small abscess was present in the space enclosed by invagination of the appendix stump. Such abscesses have been noted at post-mortem examination soon after appendicectomy for acute disease, and it seems likely that they occur in many more instances never recognized. Cases have also been recorded where such an abscess ruptured into the abdomen, with the appearance of a ruptured viscus.

The rarity of intussusception is interesting in view of the "tumour" which must frequently be created by the oedema surrounding the invaginated and infected appendix stump on so many occasions. The infective element seems important, as no case has been recorded following a "cold" appendicectomy.

These possibilities give support to the view that, except where the stump is friable and insecure, careful ligation alone may be wiser than the added purse-string.

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