Technical methods

Discussion

The preparations obtained by this method present the cells in random orientation, so that the profiles seen on electron microscopy fulfil the first criterion for morphometric analysis; quantitative studies can therefore be made of morphological changes that take place during the maturation of monocytes into macrophages. Reports have indicated that mononuclear phagocyte maturation is disordered in patients with malignant disease, and a morphometric analysis is required to define ultrastructural changes. Our method could be adapted for study of the specific effects of soluble factors, and, therefore, the presence of lymphocytes in the culture would need to be considered. Cytochemical and functional assays could also be carried out on the cultured macrophages, lymphocytes being removed if necessary by density gradient centrifugation.

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References


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Donor screening for antibody to hepatitis B surface antigen

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The acute shortage of hepatitis B surface antigen (HBsAg) immunoglobulin has prompted an investigation into the most practical methods of screening blood donors for the presence of antibody suitable for harvesting. Several methods were explored in a preliminary study of 1203 random donor sera.

Agarose diffusion is inexpensive (about $1/p per test) and easy to perform but for our purposes slow, not quantitative, and too insensitive (minimum level detected: about 20 IU/cm³). Reverse radioimmunoassay requires overnight incubation if consistent results are to be achieved, and although it is sensitive (minimum about 1 IU/cm³), it is too expensive for large scale screening (23 p per test). A commercial haemagglutination test (Serodia) is sensitive (minimum <1-0 IU/cm³) and easy to perform, but, at over 30 p per test, it too is uneconomical.

Miniaturised haemagglutination techniques in Terasaki plates have been well documented and have been in use in this centre for some years. A further modification has now been evolved that is simple to perform, gives easily read results within 30 minutes, is inexpensive at 3 p per test, like the original commercial method, and can also detect <1-0 IU/cm³ and be used for measurement.

Material and methods

The Serodia commercial passive haemagglutination kit was used (Diamed Diagnostics, Bootle, Merseyside), which uses fixed chicken cells coated with purified inactivated HBsAg. The freeze dried cells were reconstituted in 0-5 cm³ kit buffer as recommended by the manufacturer, then diluted with a further 0-5 cm³ kit buffer.

Test sera were diluted 1/40 in phosphate buffered saline in a World Health Organisation Tendix plate (Appleton-Woods, Birmingham, England) using a Compupet (General Diagnostics, Eastleigh, Hants, England), and then 5 μ of each diluted serum was transferred to a well of a Terasaki plate.

Diluted test cells (5 μl) were added to each well using a Hamilton multidispenser (Pierce and Warner, Chester, Cheshire). The plates were centrifuged for 60 seconds at 200 g in a Beckman TJ-6 centrifuge. They were then placed at room temperature on a light box angled at 45° and left for 10 minutes before reading.

A positive control (23 IU/cm³) obtained from Blood Products Laboratory, Elstree, Herts, England) was titrated with test and control cells in the range 1/40-1/20480, and a 1/10 negative control was put up without titration.

A positive test is characterised by a clear disc of cells at the bottom of the well, a negative test by a discrete crescent. Differentiation was very clear. After the preliminary study a further 4165 random sera were screened by this method alone.
Results and conclusion

A total of 5368 donors were screened. Fourteen of these (one in 383) had >10 IU/cm², the minimum level required by the Scottish Blood Transfusion Service, and 11 (one in 488) had >15 IU/cm², the level currently required by the Blood Products Laboratory for production of immunoglobulin. Our results were independently confirmed by the Blood Products Laboratory.

The method provides an easy, rapid, and economic way of screening large numbers of donors to find those with antibodies to HBsAg. These donors could then be further considered for possible plasmapheresis, with or without boosting of the antibody by hepatitis B vaccine. The method also provides a ready means, both of screening medical and other staff who may have been exposed to hepatitis B virus before vaccination and of monitoring the progress of any vaccination undertaken.

References


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Letters to the Editor

Clostridium difficile and Clostridium perfringens in upper gut of infants with protracted diarrhoea

Although the role of Clostridium difficile in the pathogenesis of colitis is well established, a possible role for C difficile in the pathogenesis of chronic diarrhoea in children without colonic disease has been suggested. On the other hand, the presence of C difficile in the small bowel has only once been reported in an elderly person with chronic diarrhoea. We report here the presence of C difficile in the upper gut of infants with chronic diarrhoea, which suggests that the organism has a potential pathogenic role in this part of the gastrointestinal tract.

We examined two groups of children to try to identify a role, if any, for clostridia in the upper gut of children: group 1 comprised 19 patients (mean age 19 months, range 2 to 48 months) presenting with protracted diarrhoea—that is, longer than two weeks—with no obvious cause at time of sampling; and group 2 comprised 10 control children (patients with coeliac disease in full clinical remission while taking a gluten free diet, mean age 39 months, range 18 to 59 months).

In all cases duodenal juice was collected under sterile conditions between the second and fourth part of the duodenum. Serial 10 fold dilutions of samples of duodenal juice were performed in an anaerobic cabinet and bacteriological cultures seeded on to both selective and non-selective media.

No clostridia were detected in any of the 10 controls, but they were found in six of the 19 patients with chronic diarrhoea: in four cases Clostridium difficile, in one case Clostridium perfringens, and in one case both, with a bacterial count ranging between 10⁸ and 10⁹ colony forming units/ml. Only one of the six patients had received treatment with antibiotics in the preceding two months.

Four patients had an eventual diagnosis of a definite gastrointestinal disease; interestingly, the remaining two were the only ones of the 19 patients who merely presented with the so-called “postgastroenteritis diarrhoea.”

To conclude, our findings, although needing further experimental evidence, suggest a potential pathogenic role for C difficile and C perfringens in the small bowel in children.

References


Expression of interleukin 2 receptor on Hodgkin's and non-Hodgkin's lymphomas and macrophages

Interleukin 2, formerly termed T cell growth factor, is an important regulator in T cell activation. Until recently it was assumed that Interleukin 2 was produced by T lymphocytes and acted exclusively on cells of the same lineage. There are now numerous reports, however, describing the expression of the interleukin 2 receptor (Tac antigen) on leukaemic (haairy cell leukaemia) and activated B lymphocytes. Applying a sensitive immunoenzymatic staining procedure (alkaline phosphatase, antialkaline phosphatase staining) on frozen tissues and cytopenepitations, we find support the relation of the Tac antigen to the B cell lineage. Moreover, almost all non-Hodgkin's lymphomas of low grade malignancy, including lymphocyte-leukaemia of the B cell type, expressed the interleukin 2 receptor, whereas those of high grade malignancy did not.

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References