All experiments in this study were carried out on 3 concentrations of bone marrow cells in order to remain in the optimal range of colony counts[32]. The chosen cell concentrations were on the linear part of the curve which expresses the relationship between the amount of plated cells and the resulting counts of colonies.

The plating of the same cell concentration before and after freezing often results in higher colony counts after freezing. The increase of numbers of CFU-c's after cryopreservation could be explained by a higher resistance of lymphocytes and stem cells against cryogenic injury. This can consequently lead to an enrichment in stem cells of the bone marrow samples after freezing. Therefore, in this study the cells were diluted in an identical way before and after freezing, and the same volume of cell suspension was used for the CFU-c assay.

It can be concluded from the data in the figure that the addition of dextran significantly ($p < 0.01$) improved the recovery provided by 5% DMSO alone. With this combination the results were at least as good as with 10% DMSO. This means that the concentration of DMSO can safely be reduced to 5%, i.e. to a lower level of toxicity. However, a further reduction of the concentration of DMSO is followed by a linear decrease of the recovery, in spite of the addition of dextran. Therefore, the combination of 5% DMSO with 9% dextran seems to be the optimum cryoprotector.

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Early B lymphocytes in man[1]

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Summary. We have confirmed in man the presence of a subpopulation of B lymphocytes which cannot reexpress their immunological receptors after challenge with antibodies. These early B lymphocytes were studied in peripheral blood, in cord blood and also, with anti-idiotypic sera, in the peripheral blood of a myeloma patient.

The reported existence of a subpopulation of mouse B lymphocytes unable to re-express surface immunoglobulins (Ig) after antigenic or antibody challenge in vivo and/or in vitro[2-4] tends to support the clonal abortion theory of B lymphocyte tolerance[5,6]. This theory postulates that, during maturation, B lymphocytes proceed through an early stage in which they are susceptible to inactivation mediated by the ligand antigen (or antibody) -surface receptor. Such inactivation could play an important role in self-tolerance in adults and neonates.

Recently, Ault and Unanue[7] have demonstrated that human IgM and IgD bearing B blood lymphocytes display a similar pattern of defective receptor resynthesis in vitro after antibody challenge, in contrast to human spleen and tonsil B cells. Human blood lymphocytes have therefore been considered to be relatively immature cells.

In this communication we present data concerning the in vitro biosynthetic capacity of human adult and cord blood lymphocytes after induction of capping-endocytosis of surface receptors by anti-Ig sera and 24 h of culture[8]. In both cases, the biosynthetic capacity decreased on passing from IgG/IgA to IgM/IgD bearing cells. Moreover, adult lymphocytes displayed a much greater resynthetic ability. This lends further support to the view that such a phenomenon is due to a subpopulation of early B lymphocytes. Furthermore, the existence of such a population was looked for

with anti-idiotypic sera in human myeloma. The anti-idiotypic sera were obtained in rabbit as previous described[9].

Blood lymphocytes were obtained from 14 healthy donors (6 males and 8 females) aged 17–49 years. In figure 1a, the percentages of Ig bearing cells, also separated according to their surface phenotype, are shown before and after resynthesis. The biosynthetic capacity in vitro constantly became greater on passing from IgM/IgD to IgG/IgA. In figure 1b, the data obtained from cord blood lymphocytes (14 cases) before and after resynthesis are shown. Here also we can see a greater ability to re-express surface receptors on the part of IgG and IgA bearing lymphocytes.

On the whole, cord blood lymphocytes displayed a resynthetic value of about 30.7% (range 10–49.8%), by comparison with the 70.8% (range 47.3–98.1%) of adult blood cells. IgD and IgM bearing lymphocytes were far more common in cord blood and their low resynthetic ability accounted for most of the total reduction in resynthesis.

In figure 2, the pattern of surface Ig expression before and after resynthesis in blood lymphocytes by an IgG myeloma in complete remission is shown. This case was selected because our previous studies with anti-idiotypic sera had revealed that all Ig bearing lymphocytes also displayed the idiotypic determinants of serum M protein on the surface[9]. Moreover, idiotypic post-resynthetic values were closely related to those obtained with Ig class-specific sera. In the figure, therefore, the post-resynthetic values of each surface Ig class also represent the post-resynthetic values of monoclonal (idiotypic) immunoglobulins in the same class. In this myeloma case, too, monoclonal IgA and IgG were resynthetized more than monoclonal IgM and IgD.

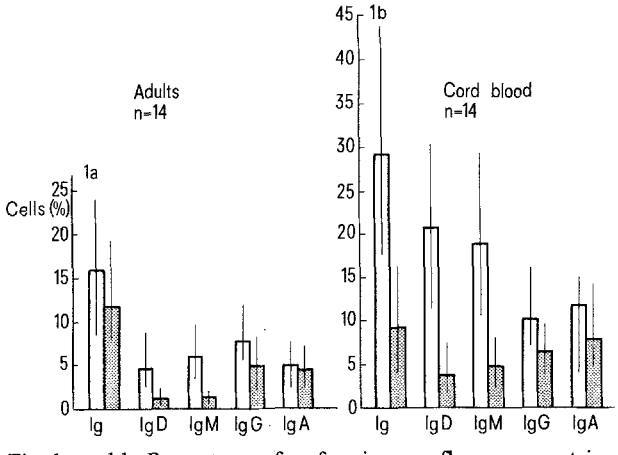

Fig. 1a and b. Percentages of surface immunofluorescence-stained lymphocytes with anti-Ig and anti-class specific rabbit antisera before (□) and after (▨) culture. Capping-endocytosis of the receptors was induced by a rabbit anti-human-Ig serum for 1 h at 37 °C (50 λ antiserum/ml, with Ig content about 10 mg/ml). Cells were then washed and allowed to stand for 24 h at 37 °C. Immunofluorescence cell percentage was measured before the culture, 2 h after challenge with anti-human-Ig serum (effectiveness of capping-endocytosis was demonstrated by values less than 2%; data not represented), and on completion of the culture (resynthesis values).

The data presented here indicate that a quota of human lymphocytes does not re-express Ig receptors as a consequence of the interaction anti Ig-surface Ig. This faulty re-expression cannot be interpreted as a result of a passive adsorption on lymphocyte membrane for the following reasons:

a) in adult subjects, defective resynthesis due to passive adsorption should be related to the serum levels of the different Ig classes (i.e. cytophilic IgG should mainly be implicated);

b) Ig serum levels in the newborn are much lower than in the adult and passive adsorption is thus less likely to occur;

c) in the case of IgG myeloma, serum M protein would be more likely to be adsorbed on the cell surface; here again, however, defective re-expression implies mostly IgM and IgD bearing cells.

As passive adsorption can be ruled out, the lower biosynthetic capacity of IgM and IgD bearing lymphocytes is an expression of their true biological properties, as suggested by experimental data in the mouse, showing that defective resynthesis does not occur when cleaning off of the lymphocyte surface is achieved by pronase or temperature-induced shedding of Ig [2,3].

Nossal and Pike[5,6] have demonstrated that tolerance can be achieved at the B cell level by showing that, during differentiation, B lymphocytes pass through an early stage in which contact with a ligand (antigen or antiserum) induces clonal abortion. This theory has been experimentally confirmed in vivo and in vitro in the mouse[2–4]. Moreover Ault and Unanue[7] have shown that human adult blood lymphocytes are more sensible to the receptor mediated inactivation than spleen and tonsil B cells; they ascribed this difference to a more precocious state of maturation.

Our data lend further support to the view that this early contact inactivation may also occur in man, on the part of younger cells, such as IgM and/or IgD bearing lymphocytes, i.e. in cord blood lymphocytes.

The occurrence of this subpopulation of early B lymphocytes may be of special interest in the immunoproliferative disorders. The fact that even in a myeloma case in remission there is a population of monoclonal lymphocytes which does not re-express surface idiotypic determinants after anti-Ig challenge in vitro, raises the hope that the abortion of the neoplastic clone as early monoclonal B lymphocytes may be possible with anti-idiotypic sera.

Fig. 2. IgG myeloma in remission: biosynthetic capacity in vitro (black areas) in B lymphocytes separated according to surface Ig classes. In this case all B lymphocytes were monoclonal, irrespective of surface Ig class, as shown by contemporary studies with anti-idiotypic sera. These sera were raised in rabbits by immunisation with purified M protein and then adsorbed on Sepharose 4B columns coupled with normal and pathological human Ig. For immunofluorescence the $(Fab')_2$ fragments were employed.

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