the growth of newly formed hybridomas.[15,39-41] The work performed on this aspect in the 1980s has culminated with the discovery that a characterized plasmacytoma/hybridoma growth factor[42,43] was identical to interleukin-6 (IL-6), which is a pleiotropic cytokine active on the differentiation of B lymphocytes.[44] The importance of adding exogenous IL-6 (or medium conditioned by IL-6–producing cells such as the P388D1 monocytic cells) to hybridoma cultures was further shown by the observation that IL-6 preferentially promoted the survival of the hybridomas secreting MoAbs.[45] The dependence to IL-6 is rapidly lost during prolonged cultures.

Another important aspect of the culture of newly formed hybridomas is the quality of the fetal calf serum used. The quality parameters are not yet defined, but it is clear that certain serum lots are much better than others for promoting the growth of newly formed B cell hybridomas. The relative performance of the serum lots should be tested in a fusion experiment rather than by their effect on the growth of myeloma or established hybridoma cells.[45]

Source of Antigen-Activated B Lymphocytes

It is quite clear that the activation of antigen-specific B lymphocytes is a parameter that is much more important than the immortalization step. As a matter of fact, myeloma cells fuse efficiently only with activated B lymphocytes that are at a differentiation stage somewhere between resting B lymphocytes and fully differentiated plasma cells.[46] Preparation of B lymphocytes at the appropriate differentiation stage is relatively easy in the murine system but represents a major problem in human hybridoma technology.

Murine Lymphocytes

The routine protocol for preparing murine B lymphocytes suitable for efficient fusion is to immunize a group of mice with the antigen of interest, determine the serum antibody level in the individual animals, select the animal showing the highest antigen-specific response, and recover the activated spleen cells 3 or 4 days after a last injection ("boost") of antigen.[15] This basic protocol has been used since the original description of the hybridoma technology and has been successful thousands of times. However, it is not unerring, and all investigators in this field have experienced failures most probably caused by the absence of antigen-activated lymphoblasts in the spleen cells recovered on the fusion day. Many of these failures may be caused by the induction in the animal immune system of a suppression/tolerance state preventing the final activation step. The possible reasons for this phenomenon are diverse and may include such parameters as seasonal variations (springtime is not very good) and the pampering of animals. One measurable parameter that may cause this phenomenon is the immunization protocol; we have observed that hyperimmunization of mice was not advisable for the preparation of a high number of antihuman red blood cells MoAbs.[47] Another parameter that is relevant to transfusion medicine concerns the optimal time period between the final antigen boost and the fusion experiment. We have previously shown that the optimal period may be shorter (2 instead of 3 to 4 days) for some antigens such as the blood group A antigen.[48]

Although several factors influencing the yield of antigen-activated B cells are still not well defined, it is clear that most problems can probably be avoided by using groups of mice and various immunization schedules.

Human Lymphocytes

The lack of a suitable and reproducible source of antigen-activated human B lymphocytes has been and still is the major difficulty in the preparation of human MoAbs by the hybridoma technology. The reasons for this are multiple. Obviously, humans cannot be immunized with antigens of interest. Furthermore, the peripheral blood was until recently (discussed subsequently) the only routinely available source of human B cells (at least to blood banks).

In vitro immunization and activation. In the 1980s, much effect was made in the development of conditions favoring the in vitro immunization and activation of human peripheral blood lymphocytes (PBL). Such systems have been described[49,50] and human MoAbs have been prepared from the B cells activated in vitro.[51-54] However, it is generally recognized that the efficiency of such systems is low compared with murine in vivo systems. In transfusion medicine-related work, several laboratories (mostly in Europe) have been successful in the preparation of human anti-Rh antibodies by using in vitro–activated (pokeweed