1. INTRODUCTION

Human B-cell tumors include a group of heterogeneous diseases with varying natural histories and responsiveness to therapy. Classic examples of B-cell tumors are Burkitt's lymphoma (BL), chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). These tumors express the conventional B-cell markers, that is, surface and/or cytoplasmic immunoglobulins. However, malignant transformation can affect precursors of the mature B-lymphocytes as exemplified by the non-T cell acute lymphoblastic leukemia (ALL). Such cases demonstrate immunoglobulin gene rearrangements and react with monoclonal antibodies to B-cell antigens. B-cell tumors, therefore, represent a spectrum of disorders extending from the immature stem cell to the most mature plasma cell of the B-lineage.

Unlike the granulocytic series where each stage of differentiation has characteristic light microscopic features, morphology is not a reliable indicator of the B-cell differentiation stage. Monoclonal antibodies to B-cell differentiation antigens have been developed, some of which are not only lineage-specific but also stage-restricted. The gain or loss of such markers in response to exogenous agents can provide an objective measure of a change in the differentiation state of the B-cells. Based on this assumption, a number of hypothetical models for B-cell differentiation have been proposed by different groups [1,2]. A hypothetical scheme of B-cell differentiation (Figure 1) is used in our institute. The range of reactivity of each antibody is based on antigen expression on fresh cells taken from patients with B-cell tumors and B-cell lines [3–7].

With the exception of Burkitt lymphoma, attempts to culture lymphomas have been mostly unsuccessful. The most common problem is the overgrowth of Epstein-Barr virus (EBV) positive lymphoblastoid cell lines from B-
lymphocyte precursors contaminating the original cultured tumor cells [8,9]. Many B-cell lines reported in the literature have been established through EBV infection [10]. Although such B-cell lines may represent the original malignant phenotype, it remains unclear whether the incorporation of the EBV in the genome altered the genetic and biological characteristics. B-cell lines which are EBV-positive show features typical of lymphoblastoid cell lines [10–13]. Such concerns make EBV-transformed cell lines unsuitable for preclinical investigation [14].

Since 1986, we have established more than ten cell lines from B-cell tumors. All of these cell lines were established without the aid of exogenous mitogens, growth factors or viral transformation and all are EBV-negative. The success rate of establishing a B-cell line is approximately 10% [15]. There are no clear predictive factors for successful establishment of such a cell line. However, tumor cells derived from serous effusions appear to have a better chance of continuous growth in vitro. In our experience, 60% of the B-cell lines were established from either a pleural effusion or ascites fluid.

When a fresh specimen is received, mononuclear cells are isolated by Lymphoprep (Nycomed Pharma AS, Oslo, Norway) density centrifugation (density of 1.077 g/ml and osmolality of 280 mOsm). Cells are washed twice