A Human X-Linked Antigen Defined by a Monoclonal Antibody

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Abstract—We have constructed hybrids between human thymocytes and the mouse thymoma BW5147. These hybrids, and others, have been used to show that the expression of a thymocyte antigen is controlled by an X-linked gene.

INTRODUCTION

Somatic cell hybrids between human and rodent cells have been used to map several genes coding for human cell-surface molecules to individual chromosomes (see, for example, references 1–3). Hybrids have also been used as immunogens for the induction of chromosome-specific antibodies. (4–6). These methods have been exploited for studying X-linked antigens. Using the former technique, Fellous et al. (7) studied the expression of the red-cell, X-linked, antigen Xga (8) on human-mouse hybrids. This work, however, has proved controversial (9). Using the latter approach Buck and Bodmer (5) immunized C3H mice with hybrids made between the L cell 1R and human lymphocytes. The antiserum raised recognized a cell-surface molecule which was preferentially expressed on B lymphocytes and was controlled by human X-linked genes. The antigen(s) recognized by this serum were designated SA-X1 (species antigen X1). Other, possibly different, antisera were subsequently raised using a similar protocol. The antigen(s) recognised were designated SA-X2 (10) and SA-X3 (11). At a recent symposium on human genetics these antigens have been renamed S10, S11, and S12 respectively. (12).
The in vitro production of monoclonal antibodies (13) has greatly simplified the study of cell-surface antigens. Several investigators have combined the technology of monoclonal antibody production with somatic cell genetics to characterize monoclonal antibodies as well as to map the genes coding for the antigens recognized (14).

We were interested in genetically characterizing and comparing two monoclonal antibodies which appear to react preferentially with human thymocytes. The first antibody was described by McMichael et al. (15) and recognizes a 44,000-dalton protein (see also reference 16), the expression of which is restricted to cortical thymocytes. The antigen has been termed HTA-1 (human thymocyte antigen 1). The second monoclonal antibody, 12E7, was described by Levy et al. (17). The 12E7 antibody was raised against T-ALL cells and reacts preferentially, but not exclusively with cortical thymocytes. Preliminary biochemical characterization implicated a 28,000-dalton protein as carrying the 12E7 determinant.

To study the genetics of the control of these antigens we have produced somatic cell hybrids between human thymocytes and a mouse thymoma. Hybrids of this type were produced in order to avoid the complication of extinction, which is often seen when cells with dissimilar phenotype are fused (18). We have used these hybrids and others to demonstrate X-linkage for the gene controlling the expression of the 12E7 antigen.

MATERIALS AND METHODS

Cells. The mouse cell BW5147, an AKR thymoma which grows in vitro and lacks the enzyme HPRT, was derived by Hyman and Stallings (19). The C3H mouse L cell derivative 1R was described by Nabholz et al. (20). Both these mouse cell lines express the $H-2^k$ haplotype and react with the monoclonal antibody 11-4.1 (21).

The human T cell related lines MOLT-4 (22) and HSB2 (23) were used as positive controls for the antithymocyte monoclonal antibodies. The primary human fibroblast cell line FS was obtained from the foreskin of a normal infant.

References to the somatic cell hybrids used in this study are given in the tables.

Growth Conditions. Cells were routinely grown in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin, and streptomycin. Suspension cells were passaged by dilution. Attached cells were transferred by treatment with a neutral solution of trypsin and EDTA (0.25% and 0.1%, respectively). To maintain the presence of the human X chromosome, hybrids were grown in medium supplemented with $10^{-4}$ M hypoxanthine, $1.0 \times 10^{-6}$