PRODUCTION OF ACTIVE ANTI-CD6 CHIMERIC IMMUNOGLOBULINS IN THE MILK OF TRANSGENIC MICE

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INTRODUCTION

Reports indicating that human proteins can be expressed in the mammary gland of transgenic farm animals, and recovered active from milk, have opened an alternative for the large-scale production of recombinant proteins, and a possible way to modify milk protein composition (1). Within this frame, expression of specific immunoglobulins in the mammary gland becomes an attractive way of intervention in animal and human health. However, functional antibodies are among the most complex molecules to find, and it could be asked if correct assembly of the four chains can be carried out successfully by the transgenic mammary gland, and active protein exported. Here we present evidence in favor of the fact that transgenic female mice can produce active mouse/human chimeric antibodies in milk.

EXPERIMENTAL PROCEDURES

The base sequences encoding for the heavy (V_H) and light (V_L) variable regions of the anti-CD6 mouse IOR-T1 monoclonal antibody were cloned by PCR (2), and inserted into two vectors containing human constant region immunoglobulin genes (Cgammal/Ck) (3). The mouse/human chimeric antibody genes were digested out from the plasmids and inserted separately into a vector containing the 5 regulatory region of the rabbit whey acidic protein gene. The two new constructions, carrying V_H-Cgammal and V_LCk inserts, were co-injected into the pronuclei of fertilized B6D2F1 mouse eggs. Transgenic animals were identified by dot and Southern blot. Milk from transgenic and control females was tested for correctly assembled heavy and ligth human constant regions, using a sandwich ELISA based on antihuman gamma 1 coating antibodies, plus an anti-human Ck-HRPO conjugate. Milk samples were also tested for specificity by indirect immuno-flourescence with peripheral blood human T lymphocytes.

RESULTS AND DISCUSSION

Single and double transgenic mice were identified. Double transgenic females were paired for milk production. Expression of assembled immunoglobulins was demonstrated in the milk of double transgenic females by ELISA. These samples were also positive for antibodies that specifically recognized peripheral blood human T lymphocytes. On the whole, our work demonstrates that functional mouse/human chimeric immunoglobulins are assembled and secreted by the mammary gland, and that idea of producing antibodies in milk for passive immunotherapy is at least technically possible. We are currently developing interbreeding experiments with single transgenic animals to find out if whole functional antibodies are produced in the milk of female offspring.

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