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THE PATHOGENETIC ROLE OF HLA-B27

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DISCUSSION

L. P. de Waal, Rapporteur

Taurog reported on disease susceptibility of HLA-B27-transgenic rats. He has observed the spontaneous development of peripheral arthritis in the majority of male rats in one of five lines of transgenic Lewis rats bearing the HLA-B2705 and human β_2-microglobulin genes. The arthritis was associated clinically with diarrhoea and histologically with a diffuse mononuclear cell inflammatory infiltrate in the gastrointestinal tract and with synovial pannus formation. Unfortunately, there were no animals expressing a control HLA class-I antigen.

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HLA Class-I-transgenic Mice as Model System to Study MHC-restricted Antigen Recognition in Man

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ABSTRACT

In order to develop a possible animal model to study HLA linked diseases of man, we established HLA-B27 transgenic mice (TGM). As aberrant and overexpression of MHC molecules can be toxic for cells, we aimed at obtaining a physiological expression of the human antigen and used a genomic 25kb Sal I fragment for embryo injection, coding for the HLA-B* 2705 heavy chain. Five independent founder mice were obtained containing varying copies of the fragment (1 to 10). RNA analysis from different tissues showed an expression pattern similar to endogenous H-2 class I genes. HLA-B27 antigen could be detected on lymphocytes derived from all five founder mice, even in the absence of human β_2-microglobulin (huβ2m). It was found that the presence of huβ2m strongly enhances HLA-B27 cell surface expression in mice with few copies of the transgene, but was not necessary for efficient and high cell surface presentation in the 10 copy line. In all HLA-B27 TGM lines, the HLA molecule functions as restriction element in anti-viral responses. In addition, we could show that T lymphocytes of the transgenic animals respond to the same HLA-B27 restricted influenza peptide as is recognized by human influenza-specific, HLA-B27 restricted cytotoxic T cell lines.

Key words: HLA-B27 transgenic mice, virus-specific cytotoxic T cell, peptide recognition.

INTRODUCTION

Because of the disease association of the human HLA-B27 antigen with ankylosing spondylitis and reactive arthritis, several groups started to generate HLA-B27 transgenic mice (TGM), in order to obtain an animal model for the HLA-B27 linked diseases in man (1, 2, 3, 4). The availability of HLA-B27-TGM might allow the study of its receptor function for viral and microbial peptides and the identification of potential enterobacterial, cross-reactive epitopes. We took advantage of our HLA-B27 cosmid clone cd2.6 (5) and used a 25 kb Sal I fragment for microinjection into outbred NMRI mice. We aimed at the integration of few copies of the transgene in order to obtain a physiological expression of the human class I restriction element. Conflicting results have been obtained in respect of the frequency of cytotoxic precursors in HLA class I TGM that could utilize the transgene product as restriction element (6, 7, 8, 9). Only one group has shown that either the HLA-Cw3 or the HLA-B27*2702 transgene can be efficiently used as restriction element for influenza or Sendai virus-specific cytotoxic T lymphocytes (CTL) (10, 1, 3). These experiments have been performed with two TGM lines, which have not been well characterized with regard to the integrated copy number of the transgene and quantitative cell surface expression. It was therefore of interest to investigate whether our five independent

lines of HLA-B*2705 transgenic mice containing one up to 10 copies would generate an anti-viral HLA-B27 restricted cytotoxic response.

Moreover, in order for HLA-TGM to present an animal model for human autoimmune diseases, it is requested that T cells of the TGM use the human restriction element in a fashion similar to human T lymphocytes. We asked the important question whether T cells of the HLA-B27-TGM recognize the same viral peptide ligand in context with HLA-B27 as human $B27^+$ T effector cells. We found that $B27^+$-TGM T lymphocytes respond to the same HLA-B27 restricted influenza peptide as has been reported for human influenza-specific, HLA-B27 restricted cytotoxic T cells (11).

MATERIALS AND METHODS

A 25 kb Sal I fragment of the HLA-B*2705 genomic clone cd2.6 (5) was used to generate transgenic mice (4, 12). Screening of the progeny was performed by the isolation of tail DNA by proteinase K digestion. 20 μg of EcoRI restricted DNA was electrophoresed on 0.7% agarose gels and transferred to nylon membrane. Hybridization with the 6.7 kb HLA-B27 EcoRI fragment was performed as described previously (13).

Indirect immunofluorescence

Total spleen or lymphnode cells were isolated and suspended in RPMI 1640/5% FCS. 1×10^6 lymphocytes were stained with supernatants of the following monoclonal antibodies: TM5 (mouse anti-HLA class I, 14) BBM.1 (mouse anti-human β2m; ATCC), and M1/42.3.9.8 (rat anti-H-2 class I; ATCC). Antibody TM5 recognizes HLA class I heavy chains regardless of the origin of β2m (14). For cytofluorometric analyses (FACSan, Becton Dickenson), lymphocytes were stained with first antibodies and subsequently incubated with FITC labeled goat anti-mouse IgG or mouse anti-rat IgG.

Generation and assay of CTL

Virus specific CTLs were generated and analysed as described (1, 3). Briefly, transgenic mice were immunized in vivo by i.p. injection of 1000 HAU of influenza virus A7X79 in 500 μl of PBS. Twenty days after priming, spleen cells were stimulated in vitro with equal numbers of virus infected and irradiated (25 GY) autologous spleen cells. After 5 days, CTLs were assayed in a 4-hr ^{51}Cr-release test on various target cells, either uninfected, or influenza infected or coated with influenza derived peptides. Infection of target lymphnode cells was started 3 hrs prior labeling, or target cells were either incubated with an HLA-B27 restricted influenza peptide derived from the nucleoprotein (NP) recognized by human influenza specific HLA-B27 restricted CTL (11) or with an $H\text{-}2D^b$ restricted NP peptide recognized by mouse influenza specific $H\text{-}2D^b$ restricted CTL (15) at two concentrations 0.1 mg/ml and 0.01 mg/ml.

RESULTS

Generation of HLA-B27 transgenic mice

A 25 kb Sal I fragment of the HLA-B*2705 cosmid clone cd2.6 (Fig. 1) was used to generate transgenic mice. This fragment covers the complete 4.3 kb HLA-B27 gene as well as its 7 kb 5′ and 14 kb 3′ flanking regions. Five independent HLA-B27 founder mice were obtained with one integration site each. The copy number ranged from 1 to 10 per cell. Two founder mice (865-11; 864-12) carried only one copy, whereas the other ones contained two copies (865-1), four copies (854-8) and ten copies (875-5), respectively.

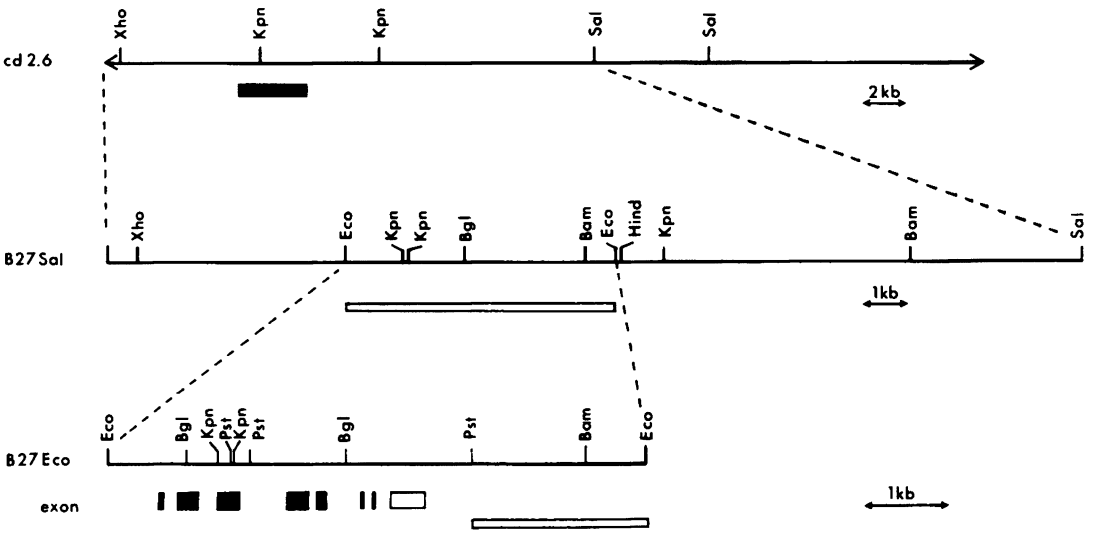

Fig. 1. The organization of the transgenic HLA-B27 Sal I fragment. The 25 kb Sal I fragment coding for the HLA-B*2705 antigen (middle line) is contained within the cosmid cd2.6 (top line, 1). On the bottom is shown the enlarged HLA-B27 gene as 6.7 kb EcoRI fragment. Closed boxes indicate the protein coding exons (1–7), and the open box shows the extent of the 3′ untranslated region. The Bgl II and the Pst I sites are only given for the EcoRI fragment.

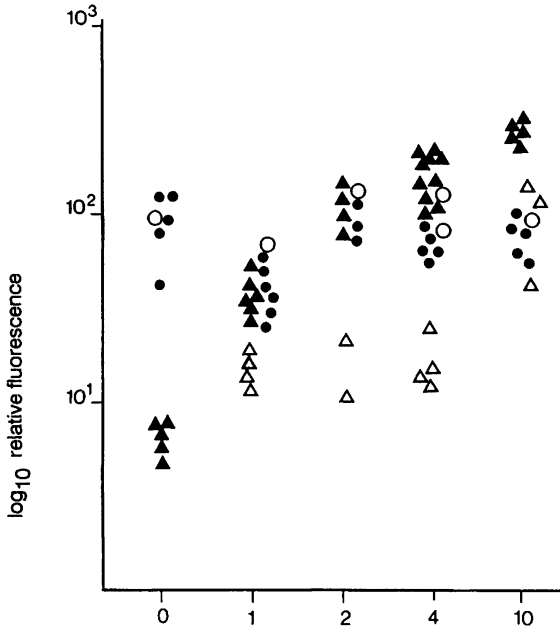

Fig. 2. Copy number and the presence of human β_2-microglobulin control cell surface expression of the human class I heavy chain in HLA-B27-TGM. Lymphocytes of the different TGM lines (1, 2, 4, and 10 copies) and of non-transgenic littermate were stained with the anti-HLA-class I monoclonal antibody TM5 (triangles) or the anti H-2-class I monoclonal antibody 42.3.9.8 (circles). The relative $\log_{10}$ fluorescence (ordinate) is compared with the integrated HLA-B27 gene copy number of the individual lines (abscissa). Each symbol presents surface fluorescence of one mouse: open symbols, HLA-B27-TGM; Closed symbols, HLA-B27/huβ2m-TGM.

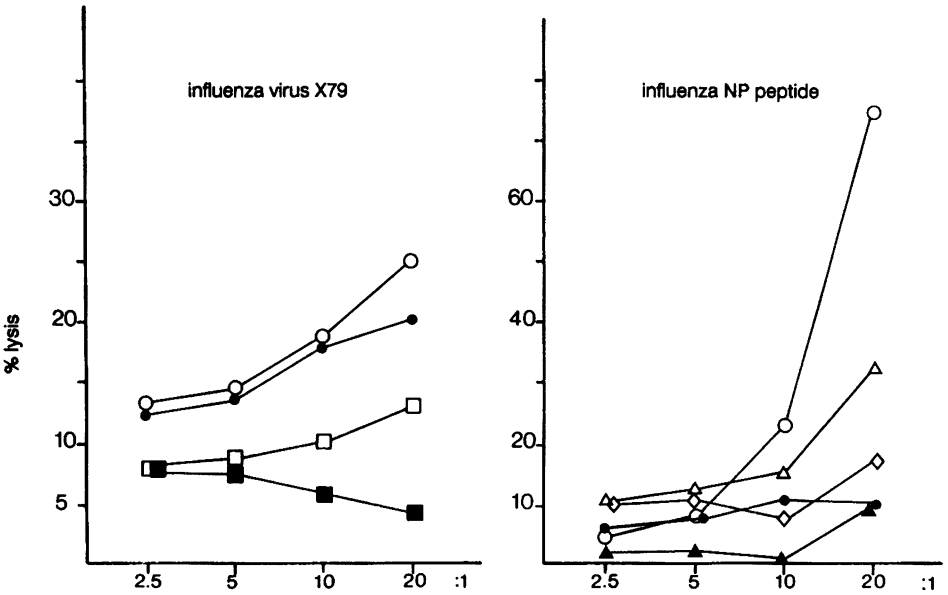

Fig. 3. HLA-B27 serves as restriction element for virus specific cytotoxic T lymphocytes in HLA-B27-TGM. *Left:* Lysis of influenza virus infected (circles) and uninfected (squares) lymphocytes of HLA-B27-TGM (open symbols) or non-transgenic littermate (closed symbols) of influenza specific CTL, measured with increasing effector to target cell ratios (abscissa) in a Cr release assay (ordinate). *Right:* Target cells were either coated with a NP peptide restricted to HLA-B27 (○/●, 0.1 mg/ml; □, 0.01 mg/ml) or with a NP peptide restricted to H-2D^b (△/▲, 0.1 mg/ml).

Tissue specific expression

To investigate tissue specific expression of the HLA-B27 transgene, RNA from spleen, liver, kidney, thymus and brain was analysed from offspring of each line by Northern blot hybridization with the HLA-B specific (13) and an H-2 class I specific probe (4). In all cases the tissue specificity of expression is concordant with that of the relevant endogenous mouse genes except in the liver, where relative low levels of HLA-B27 mRNA were detected. We found the same expression pattern in human tissues with little HLA-class I transcripts in human liver (12). Transcription in one-copy mice was approximately ten times lower than in ten-copy mice. The latter showed an HLA mRNA level similar to that of the human B cell line LG2.

Cell surface expression of HLA-B27 in transgenic mice

We analyzed all TGM lines for cell surface expression of the HLA-B27 heavy chain on splenocytes and lymph node cells. We found that all TGM lines express the HLA-B27 antigen on the cell surface (Fig. 2). The amount detected on lymphocytes correlated with the HLA-B27 gene copy number. Even mice containing only one HLA-B27 gene, could be stained with anti HLA class I reagents. Coexpression of human β_2m increases cell surface expression of the HLA-B27 heavy chain. All TGM founder mice were backcrossed to huβ2m-TGM, and singly and doubly transgenic offspring were analysed by FACS (Fig. 2). The level of HLA-B27 cell surface fluorescence was 5–10 times greater for lymphocytes from doubly transgenic mice. Only the presence of huβ2m allowed efficient cell surface representation of HLA-B27 in TGM expressing HLA-B27 mRNA at the same relative level as endogenous H-2 class I. TGM containing 10 copies of the transgene, had as much HLA-B27 molecules on the cell surface as mouse H-2 class I antigen.

Immunological functions of HLA-B27 in transgenic mice

The availability of HLA-B27-TGM allowed us to investigate the immunological function of a single HLA class I restriction element in isolation from the complexity of the human MHC. We could show that our HLA-B27-TGM carrying the HLA-B*2705 subtype were capable of using the HLA-B27 antigen as a restriction element for virus-specific T cell responses (4, and Fig. 3). As this is the case, we then addressed the question whether the TGM T cell repertoire recognizes similar peptides in context with the HLA-B27 antigen as human T lymphocytes (11). We tested anti-viral CTL obtained from 854-8/huβ2m-TGM after priming with influenza X79 virus in vivo, for recognition of the HLA-B27 restricted NP peptide. We could show that the anti-viral CTL also lyse lymphocytes of HLA-B27-TGM coated with the specific NP peptide. No lysis was observed with peptide coated cells of non-transgenic mice or when incubated with a control peptide (Fig. 3).

DISCUSSION

Although several groups described HLA-class I transgenic mice, the HLA-B27-TGM lines obtained with the 25 kb Sal I fragment are unique. First, in all lines we could detect the transgenic molecule by staining with a variety of anti-HLA-class I monoclonal antibodies. To our knowledge, no other TGM lines with one or few HLA-class I gene copies integrated, have been reported to show cell surface expression of the transgene (2, 16, 17). In addition, only the HLA-B27-TGM lines described here, show a level of transgene expression which increases with the number of integrated HLA-B27 copies. Perhaps sequences flanking the HLA-B27 gene are necessary for efficient, copy-number dependent, integration site independent transcription of the HLA-B gene (16). We also show that the frequency of cytotoxic precursors that utilize the HLA-B*2705 molecule as restriction element is rather high in all five HLA-B27-TGM lines and can be detected in bulk cultures, similar to the results obtained with a HLA-B*2702 and a HLA-Cw3-TGM line (1, 10). The reason why the HLA-A2 and HLA-B7-TGM fail to show a good HLA-restricted virus response is not clear yet. In addition, we found that TGM T lymphocytes respond to the same HLA-B27 restricted influenza peptide as human influenza-specific, HLA-B27 restricted cytotoxic T cells. This result indicates that the major epitope recognized by HLA-B27 restricted influenza specific CTL is not altered by species-specific differences in the T cell repertoire. In conclusion, our results demonstrate the utility of HLA-B27-TGM to define pathogen-specific epitopes presented by the HLA-B27 antigen. Moreover, they encourage the hope that mice transgenic for HLA-B27 may contribute towards establishing a model system for HLA-B27 associated disease studies.

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