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ORIGINAL ARTICLE

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# MUC1-specific immune responses in human MUC1 transgenic mice immunized with various human MUC1 vaccines

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**Abstract** Analyses of MUC1-specific cytotoxic T cell precursor (CTLp) frequencies were performed in mice immunized with three different MUC1 vaccine immunotherapeutic agents. Mice were immunized with either a fusion protein comprising MUC1 and glutathione *S*-transferase (MUC1-GST), MUC1-GST fusion protein coupled to mannan (MFP) or with a recombinant vaccinia virus expressing both MUC1 and interleukin-2. Mouse strain variations in immune responsiveness have been observed with these vaccines. We have constructed mice transgenic for the human *MUC1* gene to study MUC1-specific immune responses and the risk of auto-immunity following MUC1 immunization. Transgenic mice immunized with MUC1 were observed to be partially tolerant in that the MUC1-specific antibody response is lower than that observed in syngeneic but non-transgenic mice. However, a significant MUC1-specific CTLp response to all three vaccines was observed, indicating the ability to overcome T cell, but to a lesser extent B cell, tolerance to MUC1 in these mice. Histological analysis indicates no evidence of auto-immunity to the cells expressing the human MUC1 molecule. These results suggest that it is possible to generate an immune response to a cancer-related antigen without damage to normal tissues expressing the antigen.

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## Introduction

The mucin MUC1 is a membrane-bound glycoprotein found on the apical surface of ductal epithelial cells where it probably protects epithelial cells and acts as a lubricant for material passing along the duct. The expression of MUC1 can be detected on the ductal surfaces of these cells in various secretory tissues such as breast, ovary, salivary glands, lungs and pancreas [21, 30]. It is a long, rigid molecule, most of its mass being made up of a tandemly repeated segment of 20 amino acids and complex glycosylation [15, 19]. In cancers of these tissues, MUC1 is over-expressed and apical localisation is lost. Equally important, MUC1 is under-glycosylated, such that antigenic epitopes on the protein core are exposed [11]. It is these exposed epitopes on the protein core that are detected by several "tumour-specific" monoclonal antibodies [11, 26]. The observation that MUC1 is both over-expressed and underglycosylated makes it an attractive antigen for immunotherapy of these forms of cancer. Over-expression, as well as the observation that expression of MUC1 by tumour cells is no longer restricted to the apical surface, makes the molecule more accessible to a MUC1-specific immune response, antibody-mediated [34, 38] as well as restricted [5, 6] and unrestricted T-cell-mediated [9].

Several vaccine formulations of human (hu) MUC1 have been produced, most relying on synthetic or recombinant peptides the sequence of which corresponds to that of the MUC1 tandem repeat [41, 13, 12]. It has been recently shown that the T cell response to MUC1 tandem repeat peptides can be greatly enhanced by coupling mannan to the recombinant fusion protein (FP) formed by linking the tandem repeat peptide to glutathione *S*-transferase. Furthermore, if the coupling of mannan is accomplished under oxidizing conditions, immunization of mice results in a primarily Th1 type of

immune response. Immunization of mice with this molecular complex stimulates a potent cytotoxic T lymphocyte (CTL) response in mice and induces the rejection of a tumour challenge with MUC1-expressing, syngeneic tumour cells [6, 4].

We have previously described a recombinant vaccinia virus that expresses the MUC1 molecule upon infection of mammalian cells. Immunization of rats [20] or mice [1] with this construct protects animals against subsequent challenge with syngeneic tumour cells expressing MUC1 but not against control tumour cells that do not express MUC1. More recently, we have reported the construction of a recombinant vaccinia virus which, upon infection of mammalian cells, expresses both the MUC1 antigen and human interleukin-2 (IL-2) [8, 10]. Immunization of C57BL/6 but not DBA/2 mice with this virus results in the generation of a detectable CTL response [10]. Nevertheless, immunization of either strain of mouse can result in the rejection of a tumour challenge with syngeneic, MUC1-expressing, tumour cells.

It has recently been reported that mice transgenic for human *MUC1* are tolerant to human MUC1 at the level of antibody production [33], in that MUC1-specific antibody production in *MUC1*-transgenic mice was about tenfold lower than that in non-transgenic mice. The experiments described in this report agree with these data but go further, in that, while antibody responses are impaired in these mice, evidence is presented for the generation of MUC1-specific CTL precursor development in response to all three tested MUC1-based vaccines.

## Materials and methods

### Recombinant vaccinia virus

A temperature-sensitive mutant of the Copenhagen strain of vaccinia virus was used. The generation of recombinant viruses was carried out, using the plasmid pTG186 as described [25] with insertion into the thymidine kinase (*tk*) locus of the vaccinia virus genome. Transfection and selection of the $tk^-$ virus was accomplished using the 143B $tk^-$ human cell line. The cDNA coding sequence for the MUC1 protein was reconstituted from fragments of genomic DNA from human mammary tumour cells [19]. The IL-2 cDNA was obtained from a cDNA library from mitogen-stimulated human peripheral blood lymphocytes. The viral P7.5 promoter contains both early and late transcriptional signals and is used to drive both MUC1 and IL-2 coding sequences. To avoid recombination between promoter copies, the MUC1 and IL-2 expression blocks were assembled in opposing orientations.

### Mannan MUC1 fusion protein (MFP)

The preparation and production of MFP have been described in detail elsewhere [6, 30]. Briefly, an insert encoding 309 base pairs (five repeats) from the variable number tandem repeat (VNTR) region of *MUC1* was subcloned into the pGEX-3X bacterial expression vector as a fusion with the sequence encoding glutathione *S*-transferase. The resulting fusion protein was purified on glutathione/agarose beads (Sigma, St. Louis) and eluted with 5 mM reduced glutathione. Mannan (Sigma, St. Louis) was oxidized to a polyaldehyde and added to the purified MUC1 fusion protein to produce mannan fusion protein, MFP.

### MUC1-transgenic mice

The plasmid DPr/pTG8186 was generated by inserting the cDNA for the MUC1 membrane-anchoring fragment, described elsewhere [19], plus 7.2 kb of *MUC1* genomic DNA into a pPolyIII plasmid [27]. This plasmid contains the *MUC1* genomic DNA sequences extending from the 5′ *Eco*R1 site to the *Pvu*I site (Fig. 1), which is approximately 7.2 kb. The *Pvu*I site is located within the sixth exon of the membrane-bound form of the *MUC1* gene. The 7.2 kb *Eco*RI/*Pvu*I genomic fragment then continues with a *Pvu*I/*Bal*I cDNA fragment. Following the *Bal*I/*Eco*RV there are *Sal*I and *Kpn*I sites and then a 127-bp fragment simian virus 40 polyadenylation signal, a *Sal*/*Xho* site, *Not*I and then *Not*I to *Pvu*I of pPolyIII. The linear fragment was excised from the pPolyIII plasmid, using the *Not*I and *Pvu*I sites. This was injected into B6SJL eggs as described elsewhere [23]. The resulting transgenic mice were identified by Southern blotting with tail DNA. Founder males were crossed with DBA/2 females. Breeding continued with transgenic males and DBA/2 females. All mice used in the experiments described in this report were housed in the animal facility at the Austin Research Institute.

### Immunization of mice

DBA/2, C57BL/6, CBA, or MUC1 transgenic mice were bred at the Austin Research Institute (Victoria, Australia). Mice aged 6–10 weeks were immunized intraperitoneally with FP or MFP (containing 5 μg FP), or recombinant vaccinia virus expressing MUC1 and IL-2 (VVTG-5058) or vaccinia virus that is thymidine kinase-negative but has no insert in the *tk* gene (VV-186; $1 \times 10^7$ pfu in 1 ml phosphate-buffered saline, PBS) weekly for 3 weeks.

### Immunohistology

Organs were removed from immunized mice and fixed in 70% paraformaldehyde overnight at room temperature. Tissues were stained with the MUC1-specific monoclonal antibody BCP-8 as described elsewhere [40]. Briefly, paraffin was removed from sections with alcohol. Endogenous peroxidase was inhibited with $H_2O_2$ and slides subsequently washed with PBS. Slides were then incubated for 1 h at room temperature in 0.5% bovine serum albumin/Dulbecco's modified Eagle's medium (BSA/DME) containing 10 μg/ml purified antibody. Excess antibody was removed by washing the slides in PBS and the slides were then incubated for 1 h at room temperatures with 0.5% BSA/DME containing a 1/50 dilution of peroxidase-coupled rabbit anti-(mouse Ig) (Dako, Copenhagen, Denmark). Excess antibody was removed by immersing slides for 10 min in PBS. Peroxidase activity was revealed by using

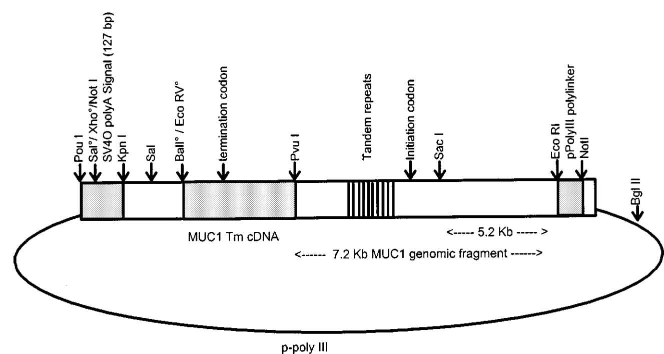


**Fig. 1** Plasmid map of pTG-8186. The plasmid was constructed as described in Materials and methods

a filtered solution of 1.5 mg/ml 3,3-diaminobenzidine (Sigma) and a final addition of 30 µl 30% $H_2O_2$. Sections were counterstained with haematoxylin for 2 min.

### Antibody analysis

Serum from immunized mice was collected 4–6 days after the third injection and tested for MUC1 antibody levels by enzyme-linked immunosorbent assay (ELISA). Blood was allowed to clot overnight at 4 °C. The serum was then removed and stored frozen at −20 °C until use. The ELISA test was performed as described elsewhere [2]. Briefly, 10 µg/ml peptide (CT1-30; PDTRPAPG-STAPPAHGVTSAPDTRPAPGSTA) containing one repeat and ten amino acids from the next repeat of the VNTR, was coated in the wells of a microtiter plate, non-specific binding was blocked with 2% bovine serum albumin, and serial dilutions of serum were added for 2 h at room temperature. After washing, sheep anti-(mouse Ig) conjugated to horseradish peroxidase (Amersham, UK) was added and incubated at room temperature and the test was developed using 50 µl 0.03% 2,2′-azino-di (3-ethylbenzthiazoline sulfonate) (Amersham, UK), 0.02% $H_2O_2$ (100 Volume, Ajax Chemical) in 0.1 M citrate buffer, pH 4.0, and incubating for 10–15 min at room temperature until the desired intensity was achieved. The absorbance was read at 405 nm in an ELISA plate reader.

### Cytotoxic T lymphocyte precursor cell (CTLp) frequency analysis

Mice immunized with one of the vaccine formulations were sacrificed 2 weeks after the final injection. For each spleen cell suspension in which CTLp frequencies were determined, a minimum of 32 replicates for each of at least six effector cell doses (ranging from $1 \times 10^3$ to $5 \times 10^5$ cells/well) were cultured in U-bottomed microtitre trays, with $5 \times 10^5$ DBA/2 spleen cells treated with mitomycin C (25 mg/ml, 1–1.5 h; Kyoma, Japan) as stimulator cells (antigen-presenting cells) in modified Eagle's medium supplemented with 10% fetal calf serum, 20 mM synthetic MUC1 peptide (C-PAH-GVTSAPDTRPAPGSTAP) and 10 U/ml recombinant human IL-2. Seven days later, each microculture was assayed for cytotoxicity by replacing 100 µl culture medium with 100 µl target cell suspension containing $10^4$ $^{51}$Cr-labelled MUC1$^+$ P815, MUC1$^+$ RMA or control P815 and RMA tumour target cells. Wells were considered to contain cytotoxic activity if they yielded a specific $^{51}$Cr release that was three standard deviations above the mean isotope release from $10^4$ effector cells cultured alone, or $10^4$ effector cells and $5 \times 10^5$ stimulators together or stimulators and peptide and recombinant IL-2 together. A linear relationship existed between the dose of effector cells, represented on a linear scale, and the frequency of negative wells on a logarithmic scale. CTLp frequencies were determined as the inverse of the responder cell dose required to generate 37% negative wells [29, 37, 13]. Each experiment was performed three or four times. Target MUC1$^+$ P815 cells have been described elsewhere [1], MUC1$^+$ RMA cells [18] were the generous gift of Dr. Joy Burchell (ICRF, London) and L929 cells were purchased from ATCC.

## Results

### VVTG-5058

We have reported previously the construction of a recombinant vaccinia virus expressing the cDNA sequences for both human MUC1 and IL-2 (VVTG-5058) [10]. This virus was shown to stimulate a CTL response in C57BL/6 mice but not in DBA/2 mice [1, 10]. Nonetheless, immunization of DBA/2 mice with VV-MUC1 was able to protect them against the growth of MUC1-expressing syngeneic tumours [1]. The data in Table 1 show that, even though bulk-culture CTL assays with spleen cells from immunized DBA/2 mice do not reveal detectable CTL activity, a significant increase in CTL precursor frequency can be detected after immunization with VVTG-5058.

### FP and MFP

A non-viral MUC1 vaccine formulation has also been produced [6, 4]. The sequence, corresponding to five tandem repeat segments of the human MUC1 sequence, has been expressed in *E. coli* as a fusion protein with glutathione *S*-transferase (GST). Mice immunized with this fusion protein stimulate weak to undetectable CTL, as detected in bulk culture, but an easily measurable increase in CTL precursor frequency [4]. If the sugar mannan is coupled to the GST part of the fusion protein under oxidizing conditions, the mannan fusion protein (MFP) stimulates a strong CTL response when injected into C57BL/6 or DBA/2 mice [5, 2]. This response can be detected by bulk-culture CTL analysis and by precursor frequency analysis (Table 1).

### MUC1 transgenic mice (TGM-8186)

It has been shown previously that immunization of mice [12, 1] or rats [20] with human MUC1 can cause the rejection of MUC1-expressing syngeneic tumour cells. A caveat with these experiments is that human MUC1 is a foreign antigen, such that an immune response may easily be possible in mice or rats, but may either be limited by self-tolerance or induce an auto-immune situation in humans. To address these questions we constructed mice transgenic for the human *MUC1* (TgM8186). Expression of human MUC1 in these mice is under the control of the human *MUC1* promoter (Materials and methods and Fig. 1). Expression of human MUC1 in these mice has been examined by immunohistology, using the monoclonal antibody BCP-8 [39]. Expression is seen in the lung bronchioles, β-islets

**Table 1** Cytotoxic T cell precursor (*CTLp*) analysis of three mouse strain responses to MUC1 immunization. The combined results of three or four experiments. All MUC1-negative control targets (P815, RMA and L929) showed undetectable CTLp levels, i.e. <1/1 000 000. Non-immunized C57/Bl6 and DBA/2 mice showed undetectable levels, i.e. <1/1 000 000 on the syngeneic targets whether MUC1-positive or -negative. *ND* not done, *MFP* MUC1-GST (glutathione *S*-transferase) fusion protein coupled to mannan

| Immunized strain | Immunogen | Target | CTLp frequency |
|---|---|---|---|
| C57Bl/6 | VVTG-5058 | RMA MUC1$^+$ | 1/60 000 |
| DBA/2 | VVTG-5058 | P815 MUC1$^+$ | 1/100 000 |
| CBA | VVTG-5058 | L929 MUC1-peptide-pulsed | <1/1 000 000 |
| C57Bl/6 | MFP | RMA MUC1$^+$ | 1/10 000 |
| DBA/2 | MFP | P815 MUC1$^+$ | 1/15 000 |
| CBA | MFP | L929 MUC1-peptide-pulsed | ND |

of the pancreas, kidney tubules and in the cells lining the stomach. The strongest staining was seen in cells of the stomach. These mice have been bred with DBA/2 mice such that they are syngeneic with the transfected murine target cell line P815-MUC1 [1].

## CTLp responses in transgenic and non-transgenic mice

To determine the whether immunization of *MUC1*-transgenic mice could generate an anti-MUC1 CTL response, TgM8186 mice were immunized intraperitoneally with MUC1-GST fusion protein, Mannan-coupled MUC1-GST fusion protein (MFP), VVTG-186 (negative control) or VVTG-5058. Cytotoxic T cell precursor (CTLp) frequency analysis was performed as described in Materials and methods. The results (Table 2) show that a significant increase in MUC1-specific CTL precursor frequency is produced in *MUC1*-transgenic mice after immunization with MFP. Fusion protein not coupled to mannan is, as has been reported elsewhere, less effective at promoting an increased frequency of CTLp. Non-transgenic DBA/2 mice immunized with MFP show a frequency three times greater than that observed in MFP-immunized transgenic mice, suggesting that some level of T cell tolerance to MUC1 exists in these transgenic mice. In contrast, in transgenic mice immunized with VVTG-5058 there is the development of an equivalent, if not greater, MUC1 CTLp frequency in transgenic mice compared to non-transgenic DBA/2 mice.

## MUC1-specific antibody responses in transgenic and non-transgenic mice

Analysis of MUC1-specific antibody responses agrees with previous observations that FP, without mannan induces a strong anti-MUC1 antibody response in non-transgenic mice, whereas MFP does not. In *MUC1*-transgenic mice, the antibody response to MUC1 generated by immunization with FP is much weaker than that observed in non-transgenic DBA mice (Fig. 2), suggesting that these mice are, at least partially, tolerant to MUC1 at the level of antibody production.

**Table 2** CTLp frequencies of *MUC1*-transgeneic and non-transgenic DBA mice after MUC1 immunization. CTLp were estimated as described in Materials and methods. Values are the average of two to four mice per immunogen. *M-GST* glutathione *S*-transferase coupled to mannan, *MFP* MUC1-GST fusion protein coupled to mannan, *FP* MUC1-GST fusion protein, *VVTG-5058* recombinant vaccinia virus expressing MUC1 and interleukin-2, *VV-186* vaccinia virus that is thymidine-kinase-negative, but has no insert in the *tk* gene

| Mice | Immunogen | CTLp P815 | CTLp P815-MUC1 |
|---|---|---|---|
| Non-transgenic DBA | M-GST | <1/1 000 000 | 1/900 000 |
| | MFP | <1/1 000 000 | **1/11 000** |
| | FP | <1/1 000 000 | **1/150 000** |
| | VVTG-5058 | <1/1 000 000 | **1/100 000** |
| | VV-186 | <1/1 000 000 | <1/1 000 000 |
| Transgenic DBA | M-GST | <1/1 000 000 | <1/1 000 000 |
| | MFP | <1/1 000 000 | **1/ 34 660** |
| | FP | <1/1 000 000 | **1/ 167 165** |
| | VV-186 | ND | <1/1 000 000 |
| | VVTG-5058 | <1/1 000 000 | **1/78 345** |

## Analyses for auto-immunity in immunized transgenic mice

Immunized transgenic and non-transgenic mice were also examined for signs of auto-immunity. TgM8186 mice were immunized intraperitoneally with MFP, $10^7$ pfu VVTG-5058 or control virus VVTG-186 ($tk^-$ but no insert). Immunized mice were kept for as long as 8 months with no overt signs of auto-immunity (weight loss, ruffling of fur). Histological sections were examined for evidence of immune or inflammatory infiltration into the areas of cells expressing the transgene. Sections of the stomach, the organ with the strongest expression of MUC1 in these mice, were examined for MUC1 expression by immunohistology using the MUC1-specific monoclonal antibody BCP-8. Serial sections were stained with haematoxylin and eosin in an effort to re-

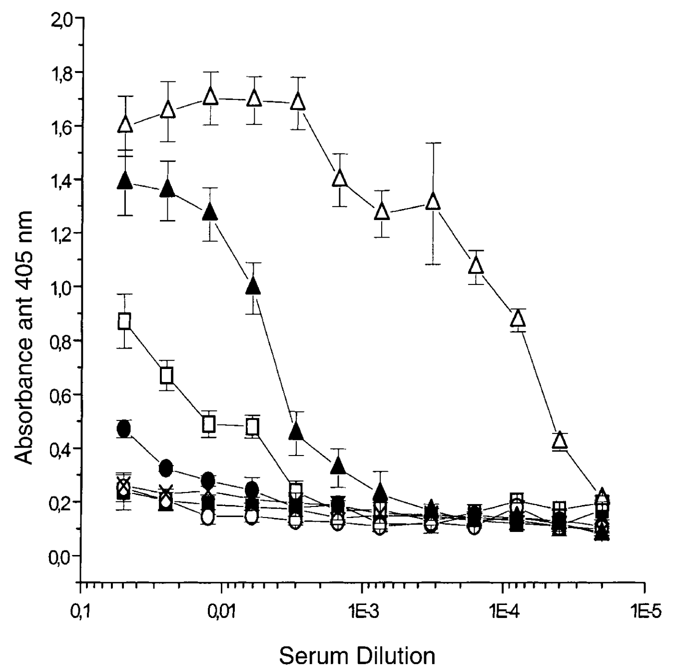


**Fig. 2** MUC1-specific antibody titres of sera from *MUC1*-transgenic or non-transgenic DBA/2 mice immunized as follows: △ non-transgenic immunized with fusion protein (FP); ▲ transgenic mice immunized with FP; □ non-transgenic mice immunized with VVTG-5058; ■ transgenic mice immunized with VVTG-5058; ○ non-transgenic mice immunized with FP coupled to mannan (MFP); ● transgenic mice immunized with MFP; **X** serum from non-immunized, non-transgenic mice. Each point represents the mean of sera from three mice ±1 standard deviation, with the exception of the normal mouse serum. The enzyme-linked immunosorbent assay was carried out as described

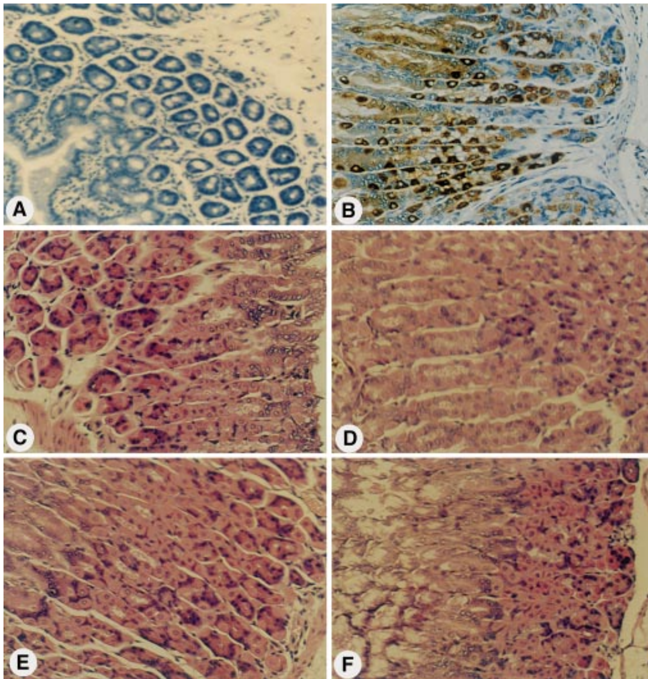


**Fig. 3A–F** Histological sections of stomach tissue from non-transgenic (**A**) and transgenic (**B–F**) mice were stained for expression of MUC1 by immunoperoxidase (**A**, **B**) as described. Stomach tissue from immunized transgenic mice (**C–F**) was stained by haematoxylin and eosin as described. **C** Non-immunized, **D** immunized with MFP, **E** immunized with VVTG-186, **F** immunized with VVTG-5058

veal any inflammation or immune infiltrate. Expression of MUC1 by the stomach cells of transgenic mice was easily revealed with BCP-8, while stomachs from non-transgenic littermates were consistently negative (Fig. 3). Nonetheless, no evidence of immune or inflammatory infiltration could be seen around MUC1-expressing cells from mice immunized with VVTG-5058 or MFP (Fig. 3). Other MUC1-expressing tissues (pancreas, kidney and lung bronchioles) also showed no such inflammation (data not shown). This evidence indicates that, while *MUC1*-transgenic mice are able to produce a cellular and an, albeit weak, antibody response to MUC1, there is no evident damage to normal tissues expressing MUC1.

## Discussion

Several vaccines incorporating the huMUC1 gene product antigen have been reported [6, 20, 10, 23, 14, 3] and have been used to immunize mice. Some strain variations in the immune response, particularly the CTL response, have been reported [5, 1, 10, 39] and (Table 1). The analysis of CTLp rather than bulk CTL assays has proven to be much more sensitive, since CTLp frequencies could be revealed in MUC1-immunized DBA/2 mice when no CTL activity could be revealed by bulk culture [6, 1, 3].

It has been important to show that huMUC1 can serve as a potent immunogen and that rejection of MUC1-bearing tumour challenge can result from immunization with huMUC1. However, in mice and rats, huMUC1 is a foreign antigen, therefore it is necessary to determine whether the recognition of huMUC1 as a self-antigen will prevent a MUC1-specific response or whether immunization with this self-antigen will result in the generation of an auto-immune response. We have constructed a line of mice transgenic for the human *MUC1* gene including the human *MUC1* promoter. Expression of human MUC1 in these mice has a distribution similar to that in humans as well as in another hu*MUC1*-transgenic line [32]. These mice have been backcrossed onto a DBA background such that the target cell line $MUC1^+$ P815 [1] can be used for CTL precursor analysis.

We show that, using three different huMUC1 vaccines, a CTL response can be induced, as demonstrated by a dramatic increase in the frequency of MUC1-specific CTL precursors. Whether these CTLp can develop into fully functional tumouricidal CTL in vivo cannot be known for certain from these data. Nonetheless, these data suggest that, despite the expression of huMUC1 as a self-antigen, and despite some evidence for tolerance to MUC1 at the level of antibody production, a MUC1-specific cell-mediated immune response can be initiated.

DBA mice immunized with a MUC1 tandem-repeat peptide fused to GST (FP) develop a strong antibody response to MUC1, whereas mice from the same strain immunized with MFP (FP coupled to mannan) develop a weak or no antibody response [4]. To test whether the *MUC1*-transgenic mice are tolerant at the level of antibody production, they were immunized with VVTG-5058, FP or MFP. The result is that *MUC1*-transgenic mice develop a much weaker antibody response to immunization with MUC1 fusion protein than do non-transgenic DBA mice. This suggests that, at the level of antibody production, these mice are, at least partially, "immunotolerant" to MUC1. A recent publication [33] shows a similar difference in antibody titre, about one order of magnitude, in *MUC1*-transgenic mice immunized with a vaccine based on *MUC1* tandem repeats. It is, nonetheless, important to acknowledge that some MUC1-specific antibody was produced in our FP-immunized *MUC1*-transgenic mice. This indicates that, while not as effectively as with CTLp development, immunotolerance to MUC1 was broken. Intraperitoneal injection of mice with VVTG-5058 (expressing MUC1 and IL-2) induces a weak antibody response in non-transgenic mice, and in *MUC1*-transgenic mice no antibody response was detectable (Fig. 2). These data are consistent with recent data from a phase I/II clinical trial in which breast cancer patients were injected with VVTG-5058 [35]. No rise in anti-MUC1 antibody titres but some cellular immune responses were observed in these patients.

Immunized mice were also analysed for histological evidence of huMUC1-associated tissue damage.

TgM8186 mice were immunized with VVTG-5058 or VVTG-186 by intraperitoneal injections. Stomach tissue was then analysed for both expression of huMUC1 and signs of a cellular infiltrate. No sign of a cellular infiltrate was seen around huMUC1-expressing tissues. Mice immunized with MFP were subjected to the same analysis and, again, no sign of an inflammatory or auto-immune infiltrate was observed. Recent data suggest that a T-cell-mediated immune response to tumour antigens can distinguish between the antigen as presented by tumour cells and the same antigen as presented by normal cells [22, 36]. While not completely consistent with the traditional model of self-tolerance by self/non-self discrimination, these data are consistent with the "danger" model of immune activation, whereby antigens, including self-antigens, are recognized by immune cells if the antigen is presented in the context of a danger signal, such as surrounding inflammation [31].

It has recently been shown that the immunization of *MUC1*-transgenic mice with dendritic cells expressing human MUC1 results in the inhibition of MUC1-expressing tumour growth [16]. In that study, mice were immunized with dendritic-cell:tumour-cell fusion products. Here we show that CTLp responses can be generated in transgenic mice by vaccine preparations that are not only suitable for injecting into cancer patients but have undergone phase I clinical trials [35, 40, 41].

We have previously published results of experiments in which mice that had been immunized with vaccinia virus expressing MUC1 [1] were able to reject implanted MUC1-expressing tumours. In a separate series of experiments, mice that were transgenic for the entire human MUC1 molecule under the control of the mouse mammary humour virus promoter were produced and bred with DBA/2 mice. These mice had a weak and inconsistent expression of MUC1 in mammary and thymus tissue. Nonetheless, like the *MUC1*-transgenic mice described in this report, they were tolerant to MUC1 immunization in that no detectable MUC1-specific antibody was produced upon i.v. immunization with MUC1-expressing virus, whereas non-transgenic littermates produced MUC1-specific IgG antibody. Injection of these immunized transgenic mice and non-transgenic littermate mice with P815 $MUC1^+$ tumour cells resulted in the subsequent rejection of tumour growth at an equivalent rate of 40%–50% (M.P. Kieny, manuscript in preparation).

The transgenic mice described in this report (human *MUC1* under the control of the human *MUC1* promoter) have recently been used in tumour rejection experiments using the MFP vaccine. As with the experiments described above, immunization of transgenic mice resulted in the inhibition of $MUC1^+$ tumour growth. This inhibition could be augmented by the co-administration of cytokines with the vaccine preparation [28].

Toxicity studies are important in examining the immune responses to MUC1 as MUC1 is a "self"-glycoprotein, occurring in normal mucin in tissues such as breast, lung, kidney, salivary gland, ovary and pancreas. Therefore, elicited anti-MUC1 responses have the potential to induce autoimmune diseases in any of these normal tissues – although this is unlikely as normal mucins are predominantly expressed intracellularly or expressed on cells lining the ducts. However, mucin peptides can be expressed on the cell surface with class I molecules [7] and therefore are accessible to T cells. Nevertheless, it is not known whether normal cells in vivo produce sufficient amounts of peptide in association with class I to be potential targets for auto-immune T cells.

It is important and encouraging to know that, despite the possible recognition of MUC1 as a self-antigen, a MUC1-specific immune response can be initiated with no sign of auto-immunity. In several MUC1-based immunotherapy phase I clinical trials so far published, no indication of an auto-immune response has been noted, while some indications of cellular immune responses have been noted [41, 17, 35, 24]. Together, these data strongly support the use of MUC1 as an immunotherapeutic for the immunotherapy of epithelial cancers.

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