Induction of viral and tumour specific CTL responses using antibody targeted HLA class I peptide complexes

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The production of cytotoxic T cells with specificity for cancer cells is a rapidly evolving branch of cancer therapeutics. A variety of approaches aim to amplify anti-tumour cytotoxic T cell responses using purified peptides, tumour cell lysates or recombinant HLA/peptide complexes in differing antigen presenting systems. Using a two-step biotin-streptavidin antibody targeting system, recombinant HLA-class I/peptide complexes were attached to the surface of B cells via the anti-CD20 B9E9-scFvSA antibody-streptavidin fusion protein. Flow cytometry with a conformation dependant monoclonal antibody to HLA class I indicated that targeted HLA-class I/peptide complexes remain on the surface of B cells in culture for periods in excess of 72 h. PBMCs were stimulated in vitro for 8–14 days using the autologous B cells as antigen presenting cells. Following a single cycle of stimulation specific cytotoxic T cell responses to targeted HLA-A2 complexes containing the M1, BMLF1 and Melan A peptides could be demonstrated by tetramer staining and Cr release assays. With the HLA-A2/BMLF1 complex up to 2.99% of CD8+ve cells were tetramer positive producing 20% lysis (E:T 10:1) of CIR-A2 target cells in an in vitro cytotoxicity assay compared to baseline levels of 0.09% tetramer +ve and 2% lysis in the unstimulated population. PBMCs from a healthy donor treated with two cycles of stimulations with targeted HLA-A2/Melan A complexes, demonstrated expansion of the melanA tetramer +ve population from 0.03% to 1.4% producing 15% lysis of Melan A pulsed target cells.

A central aim of cancer immunotherapy is the induction of effective cytotoxic T cell (CTL) activity that recognises HLA class I/peptide complexes that are either specific to or over-represented on tumour cells (Rosenberg, 1996). There is increasing evidence that low levels of CTLs specific for ‘tumour’ peptides are present in a number of malignancies (Pittet et al, 1999), however the magnitude of these pre-existing responses frequently appears to be insufficient for effective in vivo activity.

The interaction between the HLA class I/peptide complex and the T cells antigen receptor is the final pathway in the expansion of CD8+ve CTLs. A range of approaches aim to reach this interaction, starting with either defined tumour associated peptide or more complex cellular based preparations. These methods include vaccination with peptides (Rosenberg et al, 1998), naked DNA (Minchell et al, 2000) or irradiated tumour cells (Chan and Morton, 1998), these systems rely on processing and presentation by native antigen presenting cells (APCs). Alternatively ex vivo expanded dendritic cells can be used either with peptide pulsing (Hsu et al, 1996; Brossart et al, 2000), loading with tumour lysate (Nestle et al, 1999) or transfected with genes encoding tumour proteins (Wang et al, 2000a). Recombinant HLA-class I/peptide complexes either immobilised on beads (Lone et al, 1998; Tham et al, 2001), incorporated into antibody based fusion proteins (Cullen et al, 1999), or as recombinant MHC tetramers (Wang et al, 2000b) have also produced effective CTL responses both in vitro and in pre-clinical models. Dendritic cells are the most effective APCs but are present in low numbers in vivo and are difficult to culture, in contrast B cells are present in large numbers, are simple to manipulate in vitro and have been demonstrated to act effectively as APCs inducing specific CTL responses in vivo (Gajewski et al, 2001).

Previously it has been demonstrated that HLA class I/viral peptide complexes targeted to B cells via an antibody delivery system can serve as effective targets for the lytic action of anti-viral CTLs (Ogg et al, 2000; Savage et al, 2002). In this current study we have used a similar system to investigate if the two-step antibody delivery system (see Figure 1) is able to produce the specific expansion of CTLs of chosen specificities from unselected populations of PBMCs.

MATERIALS AND METHODS

Antibodies

The B9E9 scFvSA fusion protein contains the single-chain variable region of the murine IgG2a anti-CD20 murine antibody B9E9 fused to the genomic streptavidin of Streptomyces avidini. The protein is...
followed by the selective proliferation of peptide specific CTLs. The delivery of recombinant biotinylated HLA class I peptide. These steps are the delivery of the anti-CD20 B9E9 sfvScSA fusion protein. Step 2 the delivering HLA-class I peptide complexes to the surface of B cells. Step 1.

Figure 1 Schematic representation of the two-step targeting system delivering HLA-class I peptide complexes to the surface of B cells. Step 1 is the delivery of the anti-CD20 B9E9 sfvScSA fusion protein. Step 2 the delivery of recombinant biotinylated HLA class I peptide. These steps are followed by the selective proliferation of peptide specific CTLs.

secreted into the periplasm of genetically engineered E. coli as monomeric subunits (43 400 Daltons) that spontaneously fold into soluble tetramers with a molecular weight of 173 600 Daltons. The four antigen-binding and biotin-binding sites of the fusion protein retain the functional capabilities of the parent molecules (Schultz et al, 2000). The FITC conjugated monoclonal antibodies used in flow cytometry were anti-MHC class I (W6/32) (Cymbus Biotechnology, Harrow, UK), anti-CD19, anti-CD80 and anti-CD86 (Dako, Ely, UK).

Cells
The CIR-A2 (Storkus et al, 1989) and Daudi (Klein et al, 1968) cell lines were grown in RPMI + 10% FCS supplemented with Penicillin and Streptomycin in a 37°C incubator with 5% CO₂. PBMCs were isolated from healthy volunteers and melanoma patients previously documented to be HLA-A2 +ve. Approximately 30 ml of venous blood was obtained by venepuncture and unfractionated PBMCs were obtained by differential centrifugation using Histopaque (Sigma, Poole, UK).

HLA-A2/peptide complex monomers and tetramers
Recombinant HLA-A2 class I molecules were obtained from ProImmune Ltd (Oxford Science Park, Oxford, UK). In brief, recombinant HLA-A2 heavy chain and beta-2 microglobulin were produced in E. coli. The functional HLA class I/peptide complex were produced by refolding around the peptide of choice and then biotinylation via the Bir A site on the HLA heavy chain (Garboczi et al, 1992; Altman et al, 1996). The peptides used in these experiments were Influenza virus M1 peptide GILGFVFTL (Gotch et al, 1987), Epstein-Barr virus (EBV) BMLF1 peptide GLCLTLVAML (Steven et al, 1997) and the modified melanoma associated Melan A peptide ELAGIGILTV (Valmori et al, 1998). The PE conjugated fluorescent HLA-A2/peptide tetramers of the same specificities used for flow cytometric analysis were also obtained from ProImmune.

Targeting of B9E9 scFvSA and HLA-A2/peptide complexes to HLA class I -ve B cells
HLA class I -ve Daudi cells were used to investigate the binding of the HLA-A2/class I peptide complexes via the B9E9 scFvSA. Cells were washed in PBS and incubated with B9E9 scFvSA diluted in PBS at 10 ug ml⁻¹ for 1 h at RT. After washing the cells were incubated with either biotinylated HLA-A2/M1 peptide complexes at 0.5 µg ml⁻¹ or PBS alone for 30 min at RT. After further washing the two groups of cells were resuspended in RPMI + 10% FCS and grown at 37°C in a 5% CO₂ atmosphere. At various time points parallel samples of cells were removed, washed and incubated for 30 min at RT with FITC conjugated W6/32, after washing the cells were analysed by flow cytometry.

The effects of B9E9 scFvSA binding on the expression of co-stimulatory molecules in PBMC B cells
PBMCs prepared by differential centrifugation were incubated with B9E9 scFvSA (10 µg ml⁻¹), IL-7 (10 ng ml⁻¹), B9E9 scFvSA and IL-7 or PBS alone for 1 h at RT. After washing the cells were placed into tissue culture media and returned to culture at 37°C. Samples were removed and double stained with PE conjugated anti-CD19 and either FITC conjugated anti-CD80 or anti-CD86 and analysed on a Becton Dickinson FACScan using FACScomp software.

In vitro immunisation protocol
PBMCs were incubated with the B9E9 scFvSA (10 µg ml⁻¹) diluted in PBS for 1 h at RT. After washing cells were incubated with the biotinylated HLA class I/peptide complex (0.5 µg ml⁻¹ in PBS) for 30 min at RT. Various controls, omitting the B9E9 scFvSA or the HLA class I/peptide complex were also performed. After washing, cells were placed into 24-well plates at 3 x 10⁶ cells per well and cultured in RPMI with 10% human AB serum. IL-7 (R and D Systems, Minneapolis, MN, USA) was added on day 1 at 10 ng ml⁻¹ and IL-2 (Chiron, Harefield, UK) was added at 10 U ml⁻¹ on day 4 and every further 3 days following the method described by Lalvani et al (1997). In the experiments with a second stimulation cycle further PBMCs were obtained and treated as above. These new cells were then mixed with the existing culture at a 1:2 ratio and the culture continued for a further 8 days.

Flow cytometry and tetramer analysis
To stain CD8 +ve cells from the PBMC culture approximately 1 x 10⁶ cells were washed in PBS, resuspended and incubated with tetramer solution for 30 min at 37°C followed by FITC conjugated anti-CD8 for 20 min at 4°C. After incubation the cells were washed, resuspended in PBS and analysed by dual colour flow cytometry. The results of flow cytometry analysis of dual stained PBMCs are shown with anti-CD8 (Y axis) and HLA-A2/M1 tetramers (X axis). Percentage figures relate to the number of tetramer positive CD8 +ve cells from the total CD8 +ve population.

Chromium release assay
Daudi or CIR-A2 cells were labelled with 2 uCi/ul of ⁵¹Cr (Amer sham Pharmacia, UK) for 1 h at 37°C then washed. Daudi cells were sequentially coated with B9E9 scFvSA and HLA-A2/M1 complexes following the method above whilst CIR-A2 cells were pulsed with the peptide of choice at a concentration of 10 uM for 1 h at 37°C. The target cells were plated at 3000 cells per well in U bottomed 96-well plates. PBMCs, media or 5% Triton X-100 were added to a final volume of 200 µl. Plates were incubated for 4 h at 37°C in a 5% CO₂ atmosphere and 50 µl of supernatant was collected and added to 150 µl of scintillant. The specific lysis was calculated as:

\[
\% \text{ lysis} = \frac{\text{experimental c.p.m.-spontaneous c.p.m.}}{\text{maximum c.p.m.-spontaneous c.p.m.}} \times 100
\]
The spontaneous release was measured from the cells incubated in media alone, the maximum release was measured from the cells incubated in 5% Triton.

RESULTS

Sequential analysis of the binding of biotinylated HLA-A2/M1 complexes to Daudi B-cell lymphoma cells via B9E9 scFvSA

The time course of the retention of the targeted HLA-A2/M1 complexes retention the HLA class I -ve Daudi cells is demonstrated in the sequential flow cytometry analyses in Figure 2. An increased fluorescence signal is demonstrated in the targeted cells which decreases with time. However a positive signal is still present at 72 h and it is probable that HLA class I/peptide complexes persist at functional levels beyond this time.

Effects of B9E9 scFvSA binding on the expression of co-stimulatory molecules in PBMC B cells

Figure 3 demonstrates that the addition of B9E9 scFvSA has no detectable effect on the expression of CD80 or CD86 on the B cells within the PBMC population. The results show the flow cytometry results for CD19 +ve cells at 3 h and 24 h. PBMCs treated with IL-7 alone or the combination of B9E9 scFvSA and IL-7 also demonstrated no change in the levels of expression of CD80 and CD86 (data not shown).

Induction of CTL activity with targeted HLA class I/peptide complexes

The ability of the antibody targeted complexes to stimulate CTL expansion was initially examined with the HLA-A2/M1 combination. In Figure 4 the tetramer analysis of the CD8+ve/HLA-A2/M1 positive cells within the unstimulated PBMCs (1), PBMCs targeted with the B9E9 scFvSA (2), and PBMCs exposed to free soluble HLA-A2/M1 complexes at 0.1 ng ml\(^{-1}\) (3) demonstrate values of 0.06% to 0.22%. In contrast the PBMCs targeted with the B9E9 scFvSA and HLA-A2/M1 complexes (4) demonstrated 2.33% tetramer positive CD8+ve cells. Using the unfractionated PBMCs at E:T 10:1, a 4 h Cr release assay, using HLA-A2/M1 coated Daudi cells as target cells, demonstrated a maximum of 10% lysis from the three control experiments but 24% from the PBMCs stimulated with HLA-A2/M1 complexes attached via the B9E9 scFvSA fusion protein.

Induced CTL responses are specific for the targeted complex

To confirm the specificity of CTL expansion, PBMCs were targeted with either B9E9 scFvSA alone (A) or B9E9 scFvSA and HLA-A2/BMLF1 complexes (B).

In Figure 5 the tetramer analysis of the PBMCs targeted with B9E9 scFvSA alone demonstrates background staining of 0.04% with HLA-A2/M1 and 0.09% with HLA-A2/BMLF1. In the Cr release assay against CIR-A2 cells either native or pulsed with M1 or BMLF1 peptide the PBMCs showed no significant activity. In contrast PBMCs targeted with the HLA-A2/BMLF1 complexes demonstrate 2.99% staining with the HLA-A2/BMLF1 tetramer but with only a background staining level of 0.01% with the HLA-A2/M1 tetramer. These cells produced 20% lysis of the...
of the five volunteers showed a greater than five-fold increase with two others showing apparent increases. In response to targeted HLA-A2/Melan A complexes greater than three-fold increases in tetramer positive cells were seen in three of four volunteers and in one of the melanoma patients.

### CTL responses to HLA-A2/M1 and Melan A can be enhanced by a repeated stimulation

Figure 6 demonstrates the CTL responses produced by two rounds of \textit{in vitro} stimulation using the same HLA-A2/peptide complex. The 4 h Cr release assay (E:T 20:1) demonstrates that PBMCs stimulated with targeted HLA-A2/M1 complexes on both day 1 and day 8 produce 14% lysis of the CIR-A2 M1 pulsed cells compared with 3% lysis of native and 6% lysis of CIR-A2 melanA pulsed cells. The increase in HLA-A2/M1 specific CTLs is shown in the tetramer series with 0.09% from unstimulated cells (1A), 0.68% after one cycle (1B) and 2.32% after two cycles (1C). In this donor similar results were seen with responses to melan A with a 15% lysis of CIR-A2 cells pulsed with the Melan A peptide and increases in tetramer staining from a background of 0.06%, 0.16% after one cycle and 1.40% after two cycles. In this experiment cells subject to one cycle of stimulation did not produce detectable activity in the Cr release assay (data not shown).

### Table 1

| Donor | HLA-A2+M1 Pre | I Cycle | BMLF1 Pre | I Cycle | MelanA Pre | I Cycle |
|-------|---------------|---------|-----------|---------|------------|---------|
| RT    | 0.21          | 1.08    | 0.54      | 0.63    | –          | –       |
| SL    | 0.04          | 0.41    | 0.28      | 0.26    | –          | –       |
| JaR   | 0.00          | 0.32    | 0.30      | 0.85    | –          | –       |
| LOB   | 0.00          | 0.01    | 0.08      | 0.31    | –          | –       |
| LL    | 0.09          | 2.20    | 0.09      | 2.99    | 0.06       | 0.26    |
| CJ    | 0.16          | 0.57    | –         | 0.25    | 0.04       | 0.17    |
| JuR   | 0.07          | 0.52    | –         | –       | 0.09       | 0.08    |
| DV    | 0.07          | 0.80    | –         | –       | 0.03       | 0.14    |
| BB*   | 0.00          | 0.22    | –         | –       | 0.41       | 1.33    |
| DB*   | 0.36          | 0.91    | –         | –       | 0.42       | 0.21    |

### Experimental Therapeutics

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**DISCUSSION**

The induction of an effective immune response against malignant cells has been an aim of cancer research for over a century. With the increasing understanding of how the immune system can differentiate between normal and malignant cells a number of cancer vaccine approaches have been examined. To date many of these have centred on the use of undefined antigens via tumour cell lysates or irradiated cells. However with the identification of a number of potential tumour epitope peptides (Boon and van der Bruggen, 1996; Vonderheide et al., 1999) and the ease of manufacture of recombinant HLA class I peptide complexes (Garboczi et al., 1992) it is now feasible to consider highly specific approaches to cancer vaccine strategies.

Purified immobilised HLA class I/peptide complexes have been shown to interact and stimulate CTLs when attached to tissue culture plates (Kane et al., 1989), chemically attached to cells (Anjua et al., 1995; Walter et al., 1997) or when coated onto beads (Motta et al., 1998). More recently antibody targeted HLA class I/peptide complexes have been demonstrated to successfully interact with CTLs to permit lysis of targeted cells in vitro (Ogg et al., 2000; Robert et al., 2000).

The ability of B cells targeted with HLA class I/peptide complexes to induce CTL responses is clearly shown in Figures 4, 5 and 6. In vitro CTL responses demonstrated by tetramer and Cr release assays were obtained when the HLA class I/peptide complexes were targeted to PBMCs pre-treated with the B9E9 scFvSA fusion protein. In contrast free HLA class I/peptide complexes produced no apparent responses indicating the requirement for either multimerisation or immobilisation of HLA class I/peptide complexes for effective CTL stimulation as previously described (Abastado et al., 1995; Motta et al., 1998). The specificity of the CTL expansion is confirmed by the results shown in Figure 5 where increases in tetramer staining and lysis of peptide pulsed target cells was only seen in response to stimulation with that specific HLA/peptide complex. The ability of this system to further increase responses by repeated stimulation is shown in the tetramer staining results of Figure 6. Here the frequencies of CTLs reactive with HLA-A2/M1 increase from 0.09% to 2.32% after a second cycle. In this donor the MelanA results of 0.06% and 1.4% show as similar pattern, with positive Cr release assays after two cycles.

The efficiency of CTL induction has previously been shown to be related to the stability and length of expression of the HLA class-I complex on the surface of antigen presenting cells (Van der Burg et al., 1996; Valmori et al., 1996; Micheletti et al., 1998). In this antibody targeting system we have aimed to optimise the time course for expression of the HLA class I complexes, by using complexes with documented long half lives and a high affinity binding system to a non-normalising B cell marker. The ability of these complexes to persist in a conformationally correct form for at least 72 h on the surface of the B cells is demonstrated in Figure 2. It is probable that functionally active levels of complexes remain on the surface of the B cells for a longer period as we have previously shown that CTLs can interact efficiently with B cells with levels of targeted HLA below that detectable by flow cytometry (Savage et al., 2002).

The data in Figure 3 demonstrates that binding of the B9E9 scFvSA to the B cells within the PBMCs either alone or in conjunction with IL-7 had no effect on the ability of antibody targeted HLA class I/peptide complexes to specifically induce the expansion of CTLs to a single specificity should prove useful for in vitro studies analysing the endogenous CTL response or the effects of other in vivo procedures. Potentially this system could also be used for the ex vivo production of CTLs for the adoptive immunotherapy of cancer and other diseases. However a vaccination procedure based on targeting HLA class I/peptide complexes to B cells in vivo via the antibody delivery system could offer significant advances in both the applicability and effectiveness of cancer vaccines.
REFERENCES

Abastado J, Lone Y, Casrouge A, Boulot G, Kourilsky P (1995) Dimerization of soluble major histocompatibility complex-peptide complexes is sufficient for activation of T cell hybridoma and induction of unresponsiveness. J Exp Med 182: 439 – 447

Alexander-Miller MA, Derby MA, Sarin A, Henkart PA, Berzofsky JA (1998) Suprasupramolecular peptide: major histocompatibility complex causes a decrease in Bc1-2 levels and allows tumour necrosis factor alpha receptor II-mediated apoptosis of cytotoxic T lymphocytes. J Exp Med 188: 1391 – 1399

Altman JD, Moss PA, Goulder PJ, Barouch DH, McHeyzer-Williams MG, Bell JL, Michael AJ, Davis MM (1998) Phenotypic analysis of antigen-specific T lymphocytes. Science 274: 94 – 96

Anjeure F, Horvath C, Cerottini JC, Luescher IF (1995) Induction of CTL in vivo by major histocompatibility complex I- peptide complexes covalently associated on the cell surface. Eur J Immunol 25: 1535 – 1540

Boon T, van der Bruggen P (1996) Human tumor antigens recognized by T lymphocytes. J Exp Med 183: 725 – 729

Brossart P, Wirths S, Stuhler G, Reichardt VL, Kanz L, Brugger W (2000) B7-1 and B7-2 and B7-3 ligand expression by tumour cells in vivo and after vaccinations with peptide-pulsed dendritic cells. Blood 96: 3102 – 3108

Chan AD, Morton DL (1998) Active immunotherapy with allogeneic tumor cell vaccines: present status. Semin Oncol 25: 611 – 622

Cullen CM, Jameson SC, DeLay M, Cottrell C, Becken ET, Choi E, Hirsch R (1999) A divalent major histocompatibility complex/IgG1 fusion protein induces antigenspecific T cell activation in vitro and in vivo. Cell Immunol 192: 54 – 62

Delon J, Bercovic N, Raposo G, Liblau R, Trautmann A (1998) Antigen-dependent and -independent Ca2+ responses triggered in T cells by dendritic cells compared with B cells. J Exp Med 188: 1473 – 1484

Gajewski TF, Fallarino F, Ashikari A, Sherman M (2001) Immunization of HLA-A2+ melanoma patients with MAGE-3 or MelanA peptide-pulsed autologous peripheral blood mononuclear cells plus recombinant human interleukin 12. Clin Can Res 7: 895s – 901s

Garboczi DN, Hung DT, Wiley DC (1992) HLA-A2-peptide complexes: refolding and crystallization of molecules expressed in Escherichia coli and complexed with single antigenic peptides. Proc Natl Acad Sci USA 89: 3429 – 3433

Gotch F, Rothbard J, Howland K, Townsend A, McMichael A (1987) Cytoxic T lymphocytes recognize a fragment of influenza virus matrix protein in association with HLA-A2. Nature 326: 881 – 882

Hsu FJ, Benike C, Fagnoni F, Liles TM, Czerwinski D, Taidi B, Engleman EG, Cullen CM, Jameson SC, Delay M, Cottrell C, Becken ET, Choi E, Hirsch R (1999) Identification of antigen-specific T cell response to isolated class I H-2 proteins and influenza peptides. J Exp Med 188: 157 – 159

Kane KP, Vitiello A, Sherman LA, Mescher MF (1989) Cytolytic T lymphocyte response to isolated class I H-2 proteins and influenza peptides. Nature 340: 157 – 159

Klein E, Klein G, Nadkarni JS, Nadkarni J, Wiggall H, Clifford P (1968) Surface Igkappa specificity on a Burkitt lymphoma cell in vivo and in cultured derivative lines. Cancer Res 28: 1300 – 1310

Lavali A, Dong L, Gog P, Pathan AA, Newell H, Hill AVS, McMichael AJ, Rowland-Jones S (1997) Optimisation of a peptide-based protocol employing IL-7 for in vitro restimulation of human cytotoxic T lymphocyte precursors. Int J Immunol 2: 65 – 77

Larsson M, Messmer D, Somersar S, Fonteneau JF, Donahoe SM, Lee M, Dunbar PR, Cerundolo V, Julkunen I, Dixon NF, Bhardwaj N (2000) Requirement of mature dendritic cells for efficient activation of influenza A-specific memory CD8+ T cells. J Immunol 165: 1182 – 1190

Loy JC, Motta I, Mottez E, Guilloux Y, Lim, A, Demay F, Ashikari A, Cottrell C, Becken ET, Choi E, Hirsch R (1997) Soluble fraction of class I human histocompatibility leukocyte antigens in the serum of liver transplant recipients. Clin Transplant 11: 98 – 103

Michelleti F, Bazzaro M, Canella A, Marastoni M, Tranillo S, Gavioli R (1999) The lifespan of major histocompatibility complex calssal/peptide complexes determines the efficiency of cytotoxic T lymphocyte responses. Immunology 96: 411 – 415

Minceff M, Tchakarov S, Zouab S, Loukine D, Botev C, Atlantova I, Georgiev G, Petrov S, Meryman HT (2000) Naked DNA and adenoviral immunizations for immunotherapy of prostate cancer: a phase II clinical trial. Eur Urol 38: 208 – 217

Motta I, Lone YC, Kourilsky P (1998) In vitro induction of naive cytotoxic T lymphocyte with complexes of peptide and recombinant MHC class I molecules coated onto beads: role of TCR/ligand density. Eur J Immunol 28: 3685 – 3695

Nestle FO, Alijagic S, Gilliet M, Sun Y, Grabbbe S, Dummer R, Burg G, Schadendorf D (1998) Vaccination of melanoma patients with peptide- or cell-based pulsed dendritic cells. Nat Med 4: 328 – 332

Ogg GS, Dunbar PR, Cerundolo V, McMichael AJ, Lemoine NR, Savage P (2000) Sensitization of tumour cells to lysis by virus-specific CTL using antibody-targeted MHC class I/peptide complexes. Br J Cancer 82: 1058 – 1062

Pittet M, Valmori D, Dunbar PR, Speiser DE, Lienard D, Lejeune P, Fleischhauer K, Cerundolo V, Cerottini JC, Romero P (1999) High frequencies of naive Melan-A/Mart-1 specific CD8+ T cells in a large proportion of human histocompatibility leukocyte antigen (HLA)-A2 individuals. J Exp Med 190: 705 – 716

Robert B, Guillaum P, Luescher I, Romero P, Mach J-P (2000) Antibody-conjugated MHC class I tetramers can target tumor cells for specific lysis by T lymphocytes. Eur J Immunol 30: 3156 – 3170

Rosenberg SA (1997) Development of cancer immunotherapies based on identification of the genes encoding cancer regression antigens. J Natl Cancer Inst 88: 1635 – 1644

Rosenberg SA, Yang JC, Schwartzentuber DJ, Hwu P, Marincola FM, Topalian SL, Restifo NP, Dudley ME, Schwarz SL, Spiess PJ, Wunderlich JR, Parkhurst MR, Kawakami Y, Seipp CA, Einhorn LH, White DE (1998) Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nat Med 4: 321 – 327

Savage P, Cowburn P, Clayton A, Man S, Lawson T, Ogg G, Lemoine N, McMichael A, Epenetos A (2002) Anti-viral Cytotoxic T cells inhibit the growth of cancer cells with antibody targeted MHC class I/peptide complexes in SCID mice. Int J Cancer 96: 561 – 566

Schultz J, Lin Y, Sanderson J, Zuo Y, Stone D, Mallet R, Wilbert S, Axworthy D (2000) A tetravalent single-chain antibody-streptavidin fusion protein for pretargeted lymphoma therapy. Cancer Res 60: 6663 – 6669

Steven NM, Annels NE, Kumar A, Leee AM, Karilla MG, Richardson AB (1997) Immediate early and early lytic cycle proteins are frequent targets of the Epstein-Barr virus induced cytotoxic T cell responses. J Exp Med 183: 1605 – 1617

Storkus WJ, Alexander J, Payne JA, Cresswell P, Dawson JR (1989) The alpha 1/alpha 2 domains of class I HLA molecules confer resistance to natural killing. J Immunol 143: 3853 – 3857

Tham EL, Jensen PL, Mescher MF (2001) Activation of antigen-specific T cells by artificial cell constructs having immobilized multimeric peptide-class I complexes and recombinant B7-Fc proteins. J Immunol Methods 249: 111 – 119

Valmori D, Fonteneau J-F, Lizana CM, Gervois N, Lienard D, Rimoldi D, Jouveille S, Contrepois L, Wands JR, Cerundolo V, Cerottini JC, Romero P (1998) Enhanced immunogenicity of peptides bound to MHC class I molecules coated onto beads: role of TCR/ligand density. Br J Cancer 78: 881 – 887

Vonderheide RH, Hahn WC, Schultz JL, Nadler LM (1999) The telomerase catalytic subunit is a widely expressed tumor-associated antigen recognised by cytotoxic T lymphocytes. Immunology 10: 673 – 679

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British Journal of Cancer (2002) 86(8), 1336 – 1342

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Walter JB, Brander C, Mammen M, Garboczi DN, Kalams SA, Whitesides G, Walker BD, Eisen HN (1997) Stimulation of human cytotoxic T cells with HIV-1-derived peptides presented by recombinant HLA-A2 peptide complexes. *Int Immunol* 9: 451 – 459

Wang TL, Ling M, Shih IM, Pham T, Pai SI, Lu Z, Kurman RJ, Pardoll DM, Wu TC (2000a) Intramuscular administration of E7-transfected dendritic cells generates the most potent E7-specific anti-tumor immunity. *Gene Therapy* 7: 726 – 733

Wang B, Maile R, Greenwood R, Collins EJ, Frelinger JA (2000b) Naive CD8+ve T cells do not require costimulation for proliferation and differentiation into cytotoxic effector cells. *J Immunol* 164: 1216 – 1222

Wherry EJ, Puorro KA, Porgador A, Eisenlohr LC (1999) The induction of virus specific CTL as a function of increasing epitope expression: responses rise steadily until excessively high levels of epitope are attained. *J Immunol* 163: 3735 – 3745

Wataya H, Kamikawaji N, Nakanishi Y, Takayama K, Hara N, Sasazuki T (2001) Quantitation of HLA-A*0201 bound tumor associated antigens on a peptide pulsed B cell line. *Hum Immunol* 62: 125 – 132

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