Characterization of T cell receptors directed against HLA-A*01 and C*07 restricted epitopes of MAGE-A3 and MAGE-A12

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Abstract

The ability of T cells that have been genetically engineered to express T cell receptors (TCRs) directed against tumor antigens to mediate tumor regression has been demonstrated in several clinical trials. These TCRs have primarily targeted HLA-A*0201 restricted TCRs, as approximately 50% of Caucasians, who represent the predominant population of patients who develop melanomas, expresses this HLA class I allele. These therapies could be extended to additional patients through the use of TCRs that target epitopes that are presented by additional class I alleles that are prevalent in this population such as HLA-C*07 and HLA-A*01, which are expressed by approximately 50% and 30% of patients, respectively. Therefore, two TCRs that recognize an epitope of MAGE-A12 in the context of HLA-C*07, as well as two TCRs that recognize an epitope of MAGE-A3 in the context of HLA-A*01 were isolated from tumor reactive T cell clones and cloned in a recombinant retroviral expression vector. Comparative studies indicated that one of the two MAGE-A3 reactive TCRs and one of the two MAGE-A12 reactive TCRs were superior to the additional TCRs in conferring transduced PBMC with the capacity to recognize a broad array of antigen and MHC positive target cells. These results provide support the use of these TCRs in cancer adoptive immunotherapy trials.

Keywords

T cell receptors; cancer/germline antigens; T cell epitopes; cancer immunotherapy

Introduction

Patients with metastatic melanoma have a poor prognosis, as their five-year survival rate is only 5% [1]. Although IL-2 can mediate complete regressions in 5–10% of patients, complete regressions are rare when using the anti-CTLA4 antibody ipilimumab [2] or PLX4032, an

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inhibitor of mutated BRAF gene products [3]. The adoptive transfer of human tumor infiltrating lymphocytes (TIL) that were selected for specific tumor reactivity lead to objective clinical responses in 50–70% of patients with metastatic melanoma, including complete regressions in approximately 10–40% of patients who were pre-treated with lymphodepleting regimens [4]. Therapies that can be more readily applied to a wider patient population, such as the use of non-selected TIL, are also currently being evaluated [5,6]; however, the clinical efficacy of TIL generated from histologies other than melanoma has not been demonstrated.

Attempts to develop more broadly applicable cancer therapies have focused on genetic modifications that confer autologous peripheral blood mononuclear cells (PBMC) with the ability to recognize antigens specifically expressed on tumor cells. The first clinical cancer trial to evaluate the efficacy of T cells whose target specificity has been re-directed towards tumor cells was carried out using cells that were genetically modified to express a TCR directed against an HLA-A*0201 restricted epitope of the MART-1 antigen [7]. This molecule is a member of the melanocyte differentiation antigen (MDA) family of antigens that are expressed on 80–90% of melanoma but not other cancer types, and are limited in their expression in normal tissues to melanocytes. In this trial, complete regressions were observed in two out of 17 melanoma patients who received autologous PBMC that were retrovirally transduced with a MART-1-reactive TCR [7]. In a subsequent trial, treatment of patients with autologous PBMC that were transduced either with a second MART-1 reactive TCR or a TCR directed against an HLA-A*0201-restricted epitope of the MDA gp100 lead to objective clinical response rates of 30% and 19%, respectively [8]. The significant skin, eye and ear toxicities observed in this trial, which presumably resulted from responses to the normal melanocytes resident in these tissues, may have been a consequence of the relatively high avidities of these TCRs.

A recent report detailed the results of a clinical trial carried out with T cells that were transduced with a TCR that recognized NY-ESO-1, a protein encoded by a member of the cancer/germline family of genes [9]. These genes are expressed in approximately one third of a variety of tumor types that include metastatic melanomas, lung, breast, prostate, bladder, and head and neck cancers, as well as 80% of synovial cell sarcoma, but are limited in their expression in normal adult tissues to the testis [10, 11]. Objective clinical responses were observed in five out of 11 melanoma patients and four out of six synovial cell sarcoma patients treated with a high-avidity TCR directed against an HLA-A*0201-restricted NY-ESO-1 epitope [9], supporting the efficacy of adoptive immunotherapy for treatment of patients other than those with melanoma.

Chimeric antigen receptors (CARs), molecules in which antibody combining sites have been genetically linked to TCR signaling domains, have also been used to target T cells to cell surface antigens that are over-expressed on tumors or that are expressed in a highly tissue-specific manner on the tumor cell surface [12–14]. In contrast to TCRs, CARs are not MHC restricted, broadening the application of cancer adoptive immunotherapies to a wider patient population. Recent reports have demonstrated the efficacy of treatment with CARs that recognize the CD19, a cell surface molecule that is expressed in B cell malignancies and limited in its expression in non-malignant tissues to normal B cells [15,16]. In an initial case report, one patient with CLL demonstrated a partial remission that lasted for 32 weeks [17], and subsequent reports have further demonstrated the efficacy of this approach in treating patients with CD19+ tumors [18,19]. While these reports are promising, there is a paucity of cell surface antigens with limited or no expression in normal tissues.

This report details the activity of T cells transduced with tumor-reactive TCRs that target an epitope of protein MAGE-A12 recognized in the context of HLA-C*07, as well as TCRs

that target an epitope of MAGE-A3 in the context of HLA-A*01. The HLA-C*07 and A*01 class I alleles represent prevalent HLA class I alleles that are expressed by approximately 50% and 30% of Caucasians, respectively [20]. Comparative analysis provided evidence for the superiority of two of the TCRs in mediating potent recognition of a broad panel of tumor targets by TCR transduced T cells. The adoptive transfer of autologous PBMC transduced with these TCRs in future clinical trials should facilitate further evaluation of the efficacy of cancer therapies targeting antigens encoded by cancer/germline genes.

Materials and methods

Cloning of TCR genes from T cell clones

The α and β chains encoding functional TCRs were isolated from two MAGE-A12 reactive, HLA-C*07 reactive T cell clones, PHIN LB831–501D/19, referred to below as 502 [21] and FM8 [22], as well as two MAGE-A3 reactive, HLA-A*01 restricted T cell clones, LAU147 CTL1 or 810/A10, referred to below as A10 [23] and NW1000 AVP-1 13–18, referred to below as 13–18. Briefly, oligo-dT was used to reverse transcribe total RNA isolated from the T cell clones into cDNA using the SMART RACE cDNA amplification kit (Clontech, Mountain View, CA). The TCR α and β chains expressed by the T cell clones were identified by carrying out 5'-RACE reactions using a primer 5'-CACTGTTGCTCTTGAA GTCC-3' that is complementary to the TCR α chain constant region and 5'-CAGGCAGTAT CTGGAGTCATTGAG-3' that is complementary to the TCR β chain constant region in combination with adaptor primers from the SMART RNA synthesis kit. After sequencing of the 5'-RACE products, full length gene products were amplified using specific primers designed to amplify the appropriate full length TCR α and β chains. The A10 TCR expresses AV12-1/BV24-1, 13–18 expresses AV12-3/BV15, 502 TCR expresses AV13-1/BV25-1, and FM8 expresses AV38-2/BV4-3.

Generation of TCR constructs and retroviral transduction

Constructs were generated to encode the appropriate TCR α chain followed by a furin cleavage site, an SGSG spacer, the P2A "self-cleaving peptide" [24] and the appropriate TCR β chain, as previous studies comparing TCR constructs generated using 2A linkers with retroviral construct containing internal promoters as well as constructs that included an internal ribosomal entry site provided evidence for the superiority of 2A constructs [25]. Amplified PCR products were cloned into the MSGV1 retroviral expression vector [26]. Two TCRs recognizing the MAGE-A3 restricted HLA-A*01 restricted epitope were designated A10 and 13–18, and two TCRs that recognized the MAGE-A12 HLA-C*07 restricted epitope were designated 502 and FM8. Transient recombinant retroviral supernatants were generated and used to transduce PBMC that were stimulated using the anti-CD3 antibody OKT3 as previously described [25].

Isolation of purified CD8^{+} and CD4^{+} T cells

Purified CD8^{+} and CD4^{+} T cells were isolated by negative selection using CD8 and CD4 T lymphocyte enrichment kits (Becton/Dickinson, Franklin Lakes, N.J.), followed by positive selection using CD8 and CD4 magnetic beads (Becton/Dickinson). The isolated CD8^{+} and CD4^{+} cells were estimated by FACS analysis to contain less than 1% contaminating CD4^{+} and CD8^{+} T cells, respectively.

Real time PCR for MAGE A family antigens and retrovirus vector

Expression of the MAGE-A12 gene product was evaluated by Q-PCR using two primers (5'- TCCGTGAGGAGGCAAGGTTC-3' and 5'- GAGCCTGCGCACCCACCAA-3') designed to specifically amplify the MAGE-A12 gene product but not other members of the

MAGE-A gene family [27] as well as a MAGE-A12 specific probe (5'-AGTGTGGGCAGGAGCTAGT GCTGCTCCG-3'). Antigen expression was determined using plasmid controls as standards for estimating copy numbers and using GAPDH for normalization. Tumor cell lines and fresh tumors expressing greater than 1,000 copies of MAGE-A12 per 10^6 copies of GAPDH were denoted as positive for MAGE-A12 expression.

The levels of transduction of the four TCRs were evaluated in some experiments using a quantitative PCR assay carried out using genomic DNA with forward (5'-TGCAAGGCATGGAAAATAC ATAACTGA-3') and reverse (5'-CACAGATATCCTGTTTGGCCCATAT-3') primers and a probe (5'-TCTCTCTGTTCCTAACCTTG-3') designed to specifically detect the MSGV1 retroviral LTR but not human endogenous retroviral sequences. Levels of the amplified products were normalized to a positive control sample of PBMC that had been transduced with a TCR directed against the NY-ESO-1:157–165 epitope that was estimated by staining with an NY-ESO-1 tetramer to contain approximately 80% transduced T cells (data not shown).

Chromium release and IFN-γ assays

Lysis of target cells by TCR-transduced T cells was evaluated using standard ^{51}Cr-release assays that were carried out as previously described [28], and the release of soluble IFN-γ from T cells that were incubated with target cells for 18 hours was evaluated as previously described [29].

Results

Cloning and characterization of genes encoding TCRs directed against epitopes of MAGE-A3 and MAGE-A12

Four T cell clones were initially identified that recognized epitopes of the MAGE-A gene family in the context of the dominant class I alleles HLA-A*01 and C*07. Approximately 30% of the melanoma patient population expresses HLA-A*01, and more than 95% of HLA-A*01+ individuals express the HLA-A*0101 sub-type, while more than 50% of melanoma patients express one of the two dominant HLA-C*07 sub-types, C*07:01 and C*07:02. The expressed TCR α and β chains were isolated from two clones, A10 and 13–18, that recognized residues 168–176 of protein MAGE-A3 (MAGE-A3:168–176) in the context of HLA-A*01. In addition, HLA-C*07 restricted TCRs recognizing a peptide corresponding to residues 170–178 of the MAGE-A12 protein (MAGE-A12:170–178) were isolated from clones 502 and FM8 T, and transcripts encoding the paired α and β chains from the four T cell clones were inserted into the MSGV1 retroviral expression vector.

The results of co-culture assays carried out with transduced PBMC demonstrated that TCR A10-transduced T cells generated high levels of IFN-γ in response to the HLA-A*01+/MAGE-A3+ tumor cell lines 397 mel, 2984 mel, and 2556 mel, cytokine levels that were between five and ten times those generated from TCR 13–18 transduced T cells (Fig. 1A). The MAGE-A3+ but HLA-A*01 negative cell lines 562, 624 and 2359 mel, as well as the MAGE-A3 negative but HLA-A*01+ renal cancer cell line 2661 RCC failed to stimulate significant levels of cytokine from either TCR A10 or 13–18 transduced T cells (Fig. 1A). The levels of cell surface expression of the transduced A10 and 13–18 TCRs could not be quantitated due to a lack of detectable staining with a specific MAGE-A3:168–176 HLA-A*01 tetramer (data not shown) as well as a lack of antibodies against the AV12-1/BV24-1 and AV12-3/BV15 TCR α and β chains that comprised the A10 and 13–18 TCRs, respectively. Nevertheless, the differences in activity of T cell transduced with the A10 and 13–18 TCR did not appear to be due to differences in the frequency of transduction with the

two TCRs, as they appeared to be equivalent (Fig. 1B). In addition, T cells transduced with the A10 TCR recognized target cells incubated with a minimum concentration of 0.5 nM MAGE-A3 168–176 peptides, a 10-fold lower concentration than required for recognition by cells transduced with the 13–18 TCR (Fig. 1 C), indicating that the A10 TCR possessed a higher functional avidity than the 13–18 TCR.

Tumor cell lines that expressed either the HLA-C*07:01 or 07:02 sub-types were then evaluated for their ability to be recognized by either TCR 502 or FM8-transduced T cells. The TCR 502 transduced T cells recognized the HLA-C*0702[+], MAGE-A12[+] tumor 624 mel as well as two HLA-C*07:01[+], MAGE-A12[+] tumors, 397 and 2359 mel, whereas FM8 transduced T cells recognized the HLA-C*07:02[+] tumor cell line 624 mel but failed to recognize 397 and 2359 mel (Fig. 1D). Neither population of transduced T cells recognized 526, 2556, or 2984 mel, MAGE-A3[+] melanoma cell lines that lacked expression of HLA-C*07, or 2630 mel, an HLA-C*07:01[+] tumor cell line that lacked expression of MAGE-A12 (Fig. 1D). A direct evaluation of cell surface expression of the 502 and FM8 TCRs could not be carried out due to lack of availability of a MAGE-A12:170–178 HLA-C*07 tetramer, as well as the lack of antibodies directed against the AV13-1 and AV38-2 α chains present in the 502 and FM8 TCRs, respectively. As shown above for the A10 and 13–18 TCRs, however, these differences did not appear to be due to differences in transduction frequencies of the two TCRs, which appeared to be similar in cells transduced with either TCR (Fig. 1B). In addition, cells transduced with the 502 TCR recognized target cells incubated with a minimum concentration of 2.5 nM MAGE-A12:170–178, a 100-fold lower concentration than that required for recognition by cells transduced with the FM8 TCR (Fig. 1E), indicating that the 502 TCR possessed a higher functional avidity than the FM8 TCR. Seven out of the seven MAGE-A12[+] melanoma cell lines that expressed either HLA-C*0701 or 0702 were recognized by TCR 502 transduced T cells (Fig. 1F). The T cells transduced with TCR FM8 recognized 1300 and 624 mel, two MAGE-A12[+] HLA-C*07:02[+] melanomas, as well as SK23 and 1909 mel, two MAGE-A12[+] melanomas that expressed both HLA-C*07:01 and 07:02, but demonstrated little or no recognition of F002, 2369 or 586 mel, three MAGE-A12[+] tumor cell lines that expressed HLA-C*07:01 alone (Fig. 1F). Neither TCR 502 nor FM8-transduced T cells recognized 1011 mel, an HLA-C*0702[+] tumor cell line that lacked expression of MAGE-A12 (Fig. 1F). These results are consistent with previous observations indicating that clone 502, which was isolated from a patient who expressed the HLA-C*07:01 sub-type, recognized MAGE-A3^{+} target cells expressing either HLA-C*07:01 or 07:02 [21]. In contrast, clone FM8, which was isolated from a patient who expressed the HLA-C*0702 sub-type, failed to recognize MAGE-A3[+] targets that expressed the HLA-C*0701 sub-type [22]. The levels of IFN-γ released by TCR 502-transduced T cells in response to the HLA-C*07:02[+] tumor cell lines 624 mel, 1300 mel and SK23 mel were also consistently higher than those released by FM8 TCR-transduced T cells (Fig. 1D and F), corroborating the results of the peptide titration assay.

Recognition of fresh un-cultured melanomas by T cells transduced with MAGE-A3 or MAGE-A12 TCRs

The T cells that were transduced with MAGE-A3 or MAGE-A12 reactive TCRs were then evaluated for their responses to enzymatic digests of fresh, un-cultured tumor cells. T cells that were transduced with TCR A10 and 13–18 recognized five of the six MAGE-A3+ and HLA-A*01[+] fresh tumors (FrTu), FrTu 3178, 2767, 2823, 2830 and 3068, but did not recognize either FrTu 2685, an HLA-A*01[+] fresh tumor that lacked expression of MAGE-A3 or the three MAGE-A3[+] fresh tumors, FrTu 2181, 3242 and 2803, that lacked expression of HLA-A*01 (Fig. 2A). The T cells transduced with TCR 502 recognized one of the four MAGE-A12[+] fresh tumors that expressed HLA-C*0701, FrTu 3068, and TCR 502 as well as FM8 transduced T cells recognized one of the two MAGE-A12[+] tumors that expressed

HLA-C*07:02, FrTu 2181 (Fig. 2B). Neither population of TCR transduced T cells recognized the HLA-C*07:01⁻ and 07:02⁻ fresh tumors 2767 or 2823, or the MAGE-A12⁻ tumors 2685, 3242 and 2803 that lacked expression of MAGE-A12. The responses to the appropriate fresh tumors were relatively low, which may in part have been due to the reduced viability of many of the these cells; however, the patterns of tumor recognition indicated that T cells transduced with these TCRs could mediate specific recognition of these targets.

Evaluation of responses to additional MAGE-A family members

Although members of the MAGE-A family possess very similar amino acid sequences, previous studies have indicated that T cells recognizing the HLA-A*01 restricted MAGE-A3:168–176 [30] and MAGE-A12:170–178 epitopes [22][21] failed to recognize similar epitopes expressed by additional MAGE-A family members. In agreement with previous results, T cells transduced with the MAGE-A3-reactive TCR A10 recognize HLA-A1⁺ target cells transfected with MAGE-A3 but failed to recognize targets transfected with MAGE-A1, A2, A4, A6, A9, A10 or A12 constructs (Fig. 3A) that encoded peptides that differed at between one and three positions from the MAGE-A3:170–178 epitope (Table I). The T cells that were transduced with either TCR 502 or FM8 recognized HLA-C*07:02⁺ targets transfected with MAGE-A12 but not MAGE-A3, A1, A2, A4, A6, A9, A10 (Fig. 3B), while T cells transduced with TCR 502 but not FM8 recognized HLA-C*07:01⁺ targets transfected with MAGE-A12 but not the additional MAGE-A family members tested (Fig. 3C).

Tumor cell lysis mediated by TCR transduced T cells

The T cells transduced with the MAGE-A3 and A12 reactive TCRs were then evaluated for their ability to lyse tumor targets. The TCR A10 transduced T cells efficiently lysed the MAGE-A3⁺, HLA-A*01⁺ tumor targets 397 and 2984 mel, whereas lower levels of lysis were observed with TCR 13–18 transduced T cells (Fig. 4A,B). The TCR A10 and 13–18 transduced T cells failed to lyse either the HLA-A*01 negative MAGE-A3⁺ target 624 mel or 2661 RCC (Fig. 4C,D), an HLA-A*01⁺ renal cancer cell line that lacked expression of MAGE-A3. T cells transduced with TCR 502 lysed the MAGE-A12⁺ tumor expressing either HLA-C*07:01 (397 mel) or C*07:02 (624 mel) (Fig. 4 A,C), whereas FM8 transduced T cells only recognized the MAGE-A3⁺ HLA-C*07:02⁺ tumor target 624 mel (Fig. 4C).

Reactivity of transduced CD8⁺ and CD4⁺ T cells

The responses of separated populations of CD8⁺ and CD4⁺ T cells transduced with the four TCRs to tumor cell targets was then evaluated, as previous results have demonstrated that high affinity class I restricted TCRs possessed significant activity in CD4⁺ T cells [31,32][33]. Highly purified CD4⁺ T cells transduced with TCR A10 containing fewer than 1% contaminating CD8⁺ T cells released low but significant levels of IFN-γ in response to MAGE-A3⁺ tumor cell line 397 mel as well as the MAGE-A3⁺ tumor cell line 1300A1 mel that was stably transfected with HLA-A*01, whereas CD4+ T cells transduced with TCR 13–18 failed to recognize antigen positive targets (Fig. 5A). Transduced CD4⁺ T cells expressing either the 502 or FM8 TCR failed to recognize the MAGE-A12⁺ and HLA-C*07⁺ tumor cell lines that were tested (Fig. 5B).

DISCUSSION

The development of efficient methods for transducing autologous PBMC with recombinant retroviral and lentiviral constructs that encode tumor-reactive TCRs has facilitated the extension of adoptive T cell immunotherapies to a broad patient population. Objective response rates of 30% and 19% were observed in melanoma patients who received autologous PBMC transduced with TCRs targeting HLA-A*0201 restricted epitopes of the

MART-1 and gp100 antigens, respectively [8]; however, many of the patients treated in this trial developed severe skin, eye and ear toxicity due to the expression of MART-1 and gp100 on normal melanocytes. In a recent trial targeting an HLA-A*02:01 restricted epitope of NY-ESO-1, melanoma and synovial sarcoma patients who received autologous TCR transduced T cells demonstrated objective response rates of 45% and 67%, respectively [9]. Normal tissue toxicity was not evident in patients enrolled in this trial, presumably due to the fact that NY-ESO-1 expression is limited to cells in the normal testis that lack HLA expression.

In the current study, TCRs were characterized that recognize epitopes of MAGE-A3 and MAGE-A12, which have been shown to be expressed in between 30 and 60% of a variety of tumors that include melanoma, bladder, esophageal and prostate cancer. The lack of tetramer staining observed in cells transduced with the MAGE-A3:168–176-reactive TCRs and the unavailability of a MAGE-A12:170–178 tetramer hampered direct evaluation of cell surface expression of the transduced TCR. Tetramer staining, however, is influenced by TCR avidity for the peptide/MHC complex affinity, and thus does not provide a direct readout of cell surface TCR expression [34]. Therefore, a direct comparison of the functional avidity of the T cells was carried out be evaluating their ability to recognize tumor targets, as well as cells pulsed with titrated doses of peptide. Results of comparisons between two TCRs that recognized an HLA-A*01-restricted epitope derived from MAGE-A3 but not from any additional MAGE family members revealed that cells transduced with the A10 TCR efficiently lysed and secrete high levels of IFN-γ in response to appropriate tumor target cells, while cells transduced with the 13–18 TCR demonstrated significantly weaker responses to these targets. In addition, cells transduced with the A10 TCR recognized target cells incubated with a 10-fold lower concentration of the MAGE-A3:168–176 peptide than those transduced with the 13–18 TCR. A similar comparison was carried out between two TCRs, 502 and FM8, directed against an HLA-C*07 restricted epitope derived from MAGE-A12 but not from any additional MAGE family members. The T cells that were transduced with TCR 502 lysed and secreted IFN-γ in response to MAGE-A12 targets expressing either HLA-C*07:01 or 07:02, two highly prevalent HLA-C*07 subtypes that are both expressed by approximately 25% of the patient population. In contrast, T cells transduced with TCR FM8 only recognized MAGE-A12$^+$/HLA-C*0702$^+$ target cells. Cells transduced with the 502 TCR also recognized HLA-C*0702$^+$ target cells incubated with a 100-fold lower concentration of the MAGE-A12:170–178 peptide than those transduced with the FM8 TCR, indicating that the 502 TCR possessed a higher functional avidity than the FM8 TCR. The levels of cytokines secreted in response to fresh, un-cultured tumor cells by T cells transduced with the MAGE-A3 and MAGE-A12 reactive TCRs were lower than those produced in response to tumor cell lines but were consistent with the patterns of HLA and antigen expression in these cells. Further evaluation indicated that TCR A10 transduced CD4$^+$ T cells generated low but significant levels of IFN-γ in response to HLA-A1 and MAGE-A3$^+$ tumor cells, indicating that this TCR possesses a relatively high avidity.

Mis-pairing of transduced TCR α or β chains with endogenous receptor chains poses potential risks to patients, as studies carried out in murine model systems have demonstrated that adoptively transferred autologous TCR-transduced T cells can lead to lethal graft versus host disease (GVHD) [35]. The observed toxicities were dependent on the particular TCR used for transduction, however, and off-target toxicity has not been observed in the nearly 150 patients who received autologous T cells transduced with TCR directed against a variety of target antigens (summarized in reference 36). In recent clinical TCR trials [4,8], TCR α and β transcripts have also been linked with a porcine teschovirus-derived P2A sequence rather than an internal ribosomal entry site, which enhanced pairing of the transduced α and β chain products [37] and reduced the GVHD observed in the murine TCR study [35]. While these observations suggest that caution may be warranted in clinical trials utilizing TCRs that have

not yet been administered to patients, it should be weighed against the urgent need to develop effective treatments for patients bearing lethal cancers.

Currently available antibodies cross-react with multiple MAGE-A family members and thus will not allow tumors that specifically express MAGE-A3 or MAGE-A12 to be distinguished from those expressing other family members [38]. The MAGE-A2, A3, A6 and A12 genes are tightly clustered in a region on the long arm of the X chromosome and are coordinately expressed in melanomas as well as additional tumor types [39]. Genes present in this cluster also appear to be expressed in a higher percentages of tumors than other MAGE-A family members that are present on different regions of the X chromosome [39], and as a result, nearly all of the tumors that stain positively with these antibodies will express MAGE-A3 and MAGE-A12. Quantitative RT-PCR assays of fresh tumor samples using primers and probes that are specific for MAGE-A3 and MAGE-A12 transcripts can also be used to confirm expression of these gene products.

The identification of TCRs that target a variety of antigens encoded by cancer/germline genes will allow the treatment of a significantly larger population of cancer patients with TCR-based therapies. An HLA-A*02:01 restricted TCR that recognizes an epitope shared by MAGE-A3 and MAGE-A9, as well as a similar epitope expressed by MAGE-A12 has recently been characterized [40] and can be used to treat approximately 20% of patients with a variety of cancer types that include melanoma, prostate, lung, bladder and head and neck cancer. Between 15 and 20% of patients with these cancers could potentially be treated with a TCR that target the HLA-C*07 restricted MAGE-A12 epitope, and approximately 10% of patients could potentially be treated with a TCR that recognizing the HLA-A*01 restricted MAGE-A3 epitope, taking into account the frequency of these class I alleles in the patient population as well as the frequency of expression of the MAGE-A3 and MAGE-A12 antigens in tumors. The identification and characterization of potent TCRs that target epitopes derived from antigens encoded by cancer/germline gene in the context of additional HLA alleles such as HLA-A*24, B*35 or B44, class I alleles that are expressed by between 10 and 20% of patients, may lead to the development of a set of reagents that could be used to treat the majority of patients bearing these common malignancies.

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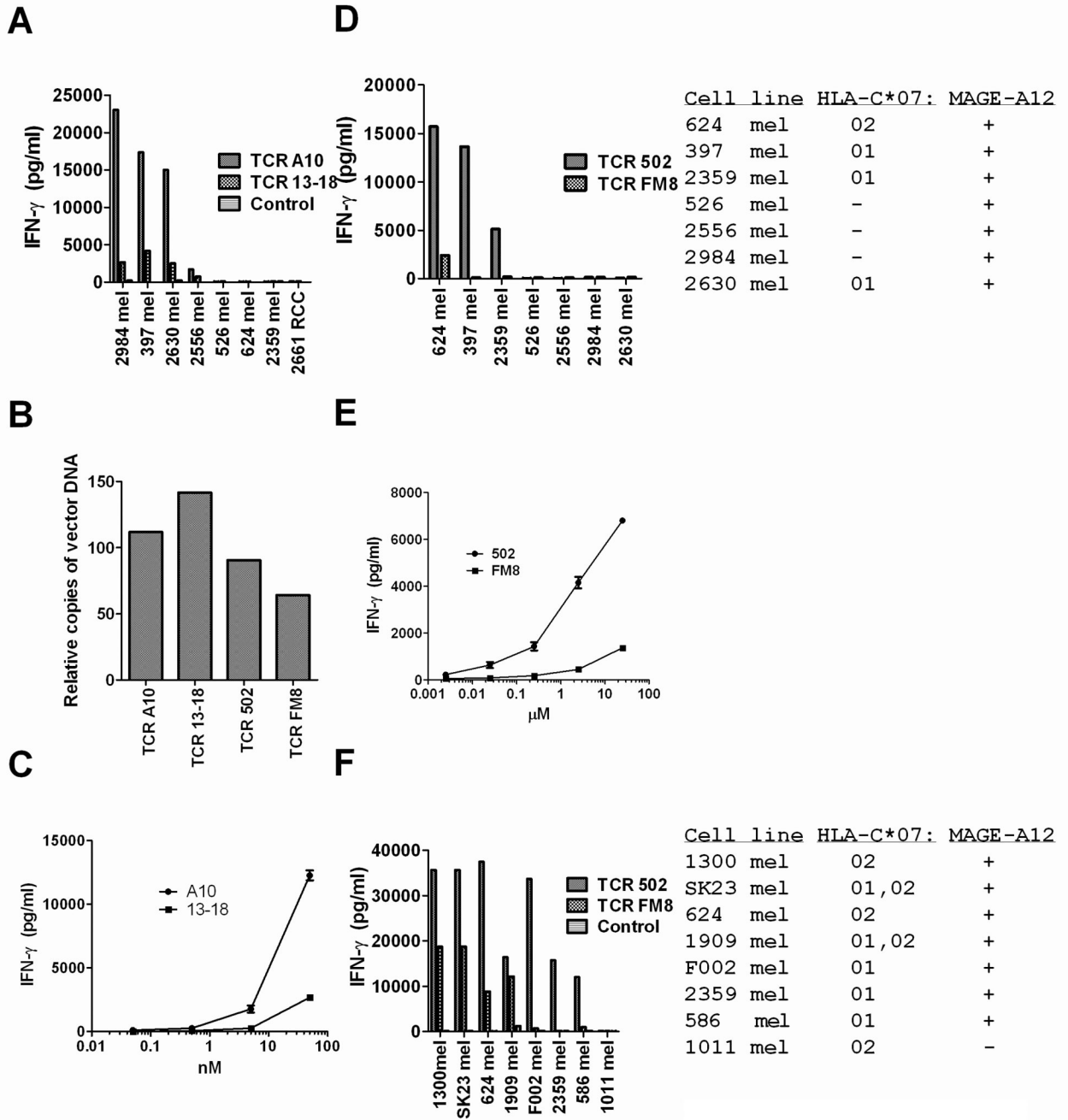
Figure 1.
Responses of TCR transduced T cells to tumor cell lines. Anti-CD3 stimulated PBMC were transduced with TCR A10 or 13–18, which recognized the MAGE-A3:168–176 HLA-A*01 restricted epitope (A–C), or with TCR 502 or FM8, which recognized the MAGE-A12:170–178 HLA-C*07 restricted epitope (B, D–F). The TCR-transduced T cells, as well as un-transduced control T cells, were evaluated for their ability to release IFN-γ in response to a panel of tumor cell lines in an overnight co-culture assay. Estimates of relative transduction frequencies of PBMC used for the experiments shown in (A) and (D) are shown in (B). Representative results from two of three independent experiments assessing responses of T cells transduced with these TCRs are presented.
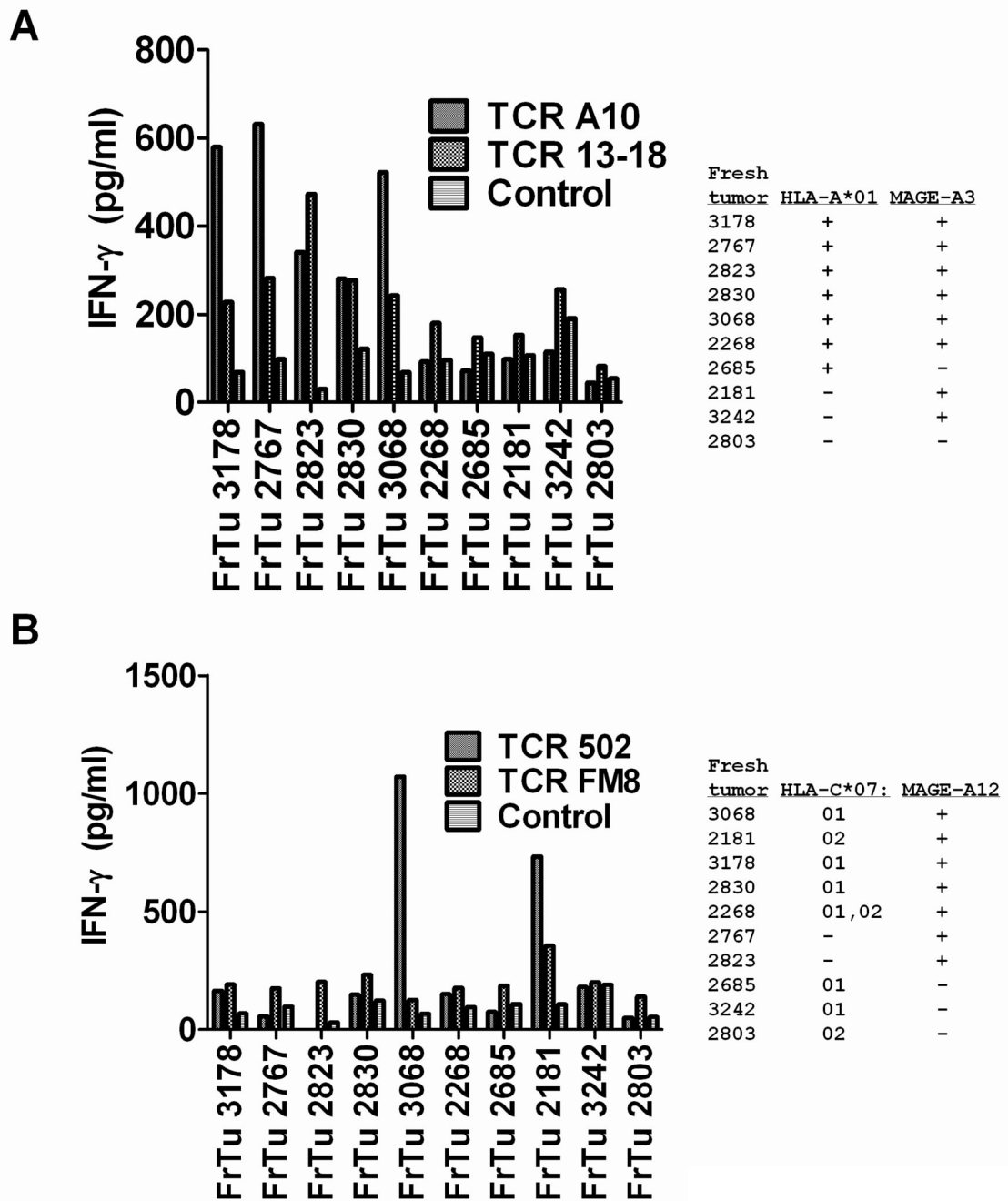
Figure 2.
Responses of TCR transduced T cells to fresh uncultured melanomas. Patient PBMC that were transduced with either TCR A10, 13–18, 502 or FM8 were assayed for their ability to release IFN-γ in response to a panel of fresh un-cultured melanomas that either did or did not express HLA-A*01 and MAGE-A3 (A) or did or did not express HLA-C*07:01 or HLA-C*07:02 and MAGE-A12 (B) in an overnight co-culture assay. In this experiment, the HLA-A*01[+]/MAGE-A3[+] tumor cell line 397 mel stimulated the release of 17,400 and 4,200 pg/ml of IFN-γ from T cells transduced with TCR A10 and 13–18, respectively, while T cells transduced with TCR 502 and FM8 released 15,700 and 2,400 pg/ml of IFN-γ , respectively, in response to the MAGE-A12[+]/HLA- C*07:02[+] tumor cell line 624.

Representative results from one of three independent experiments assessing responses of T cells transduced with these TCRs are presented.
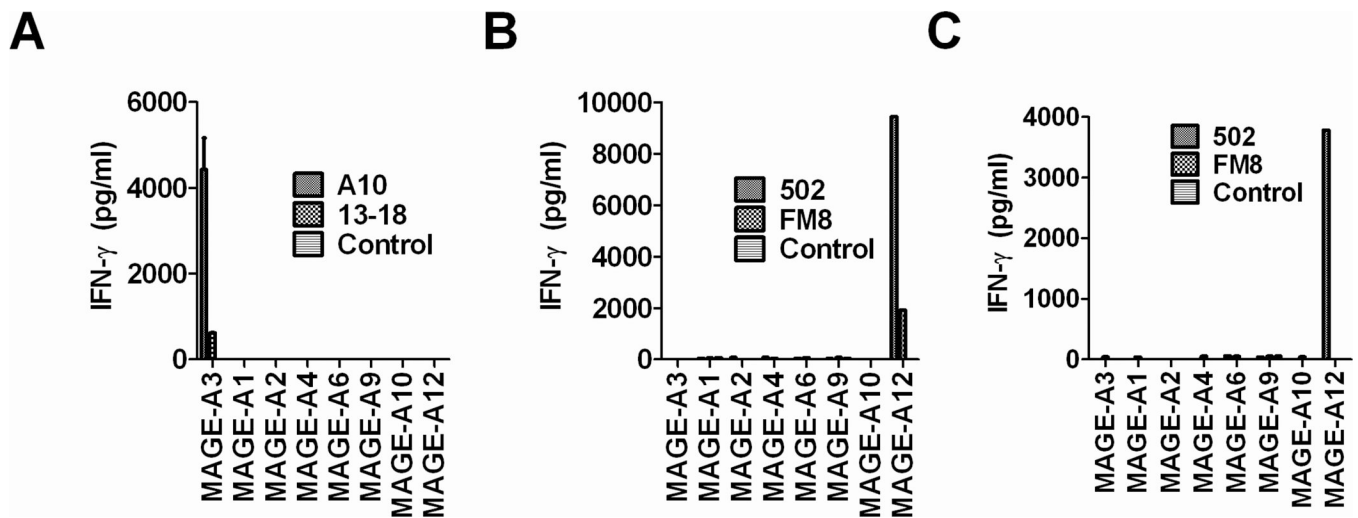
Figure 3.
Ability of TCR transduced T cells to recognize target cells transfected with MAGE-A gene family members. The monkey kidney cell line COS-7 was transiently transfected with either HLA-A*01 (A), C*07:01 (B) or C*07:02 (C) plus either MAGE-A3, A1, A2, A4, A6, A9, A10 or A12 overnight. The following day T cells transduced with either TCR A10, 13–18 or un-transduced control cells (A) or TCR 502, FM8 or un-transduced control cells (B and C) were added and the release of soluble IFN-γ was evaluated following an overnight co-culture by ELISA.
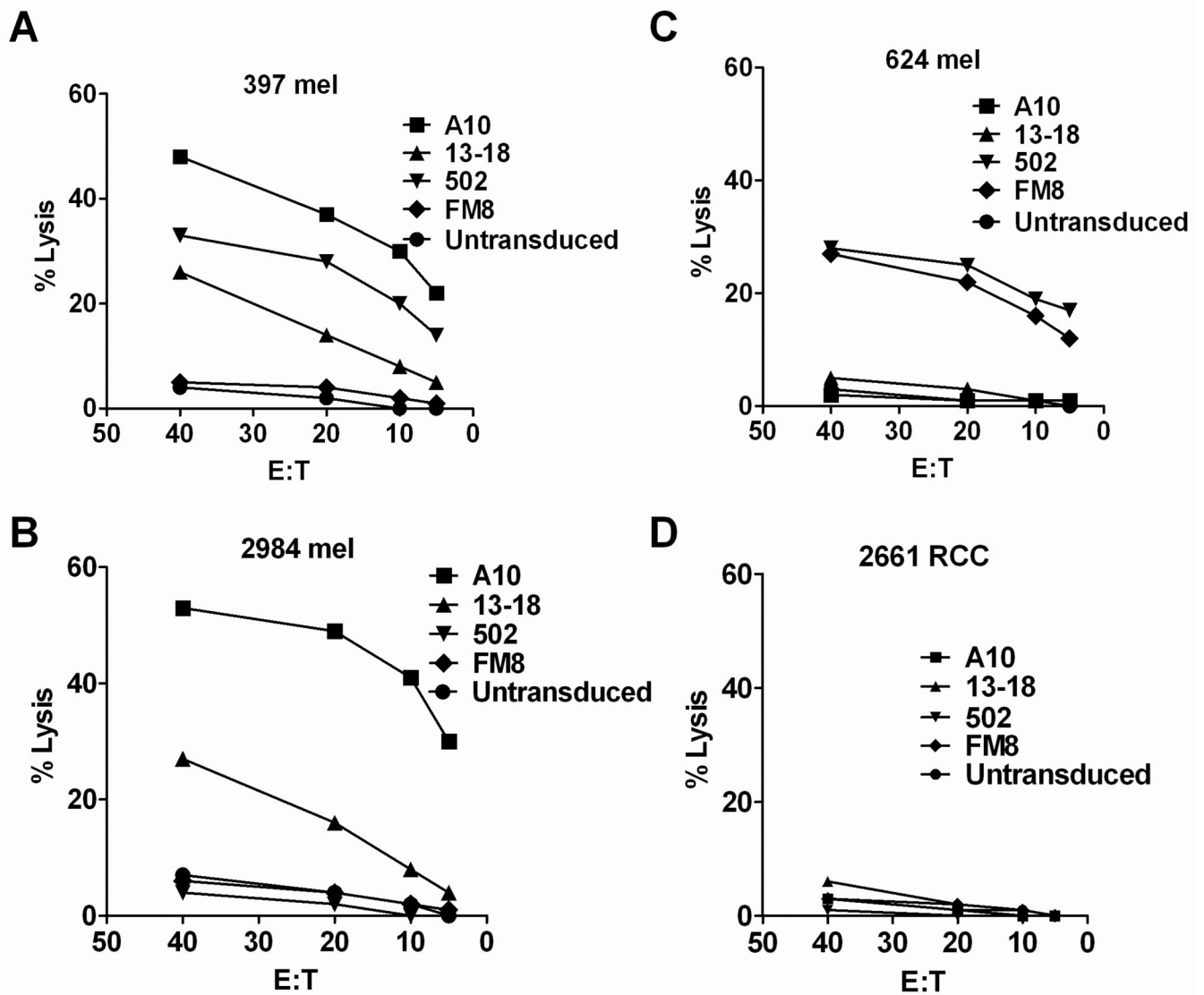
Figure 4.
Cytolytic activity of TCR transduced T cells. Following stimulation with anti-CD3 antibody, PMBC from a single donor were transduced with TCRs that recognize the MAGE-3 and MAGE-A12 tumor antigens. Thirteen days after stimulation, transduced T cells were incubated with the indicated tumor targets in a standard 4 hour ^{51}Cr release assay. Representative results from one of two independent experiments are presented.
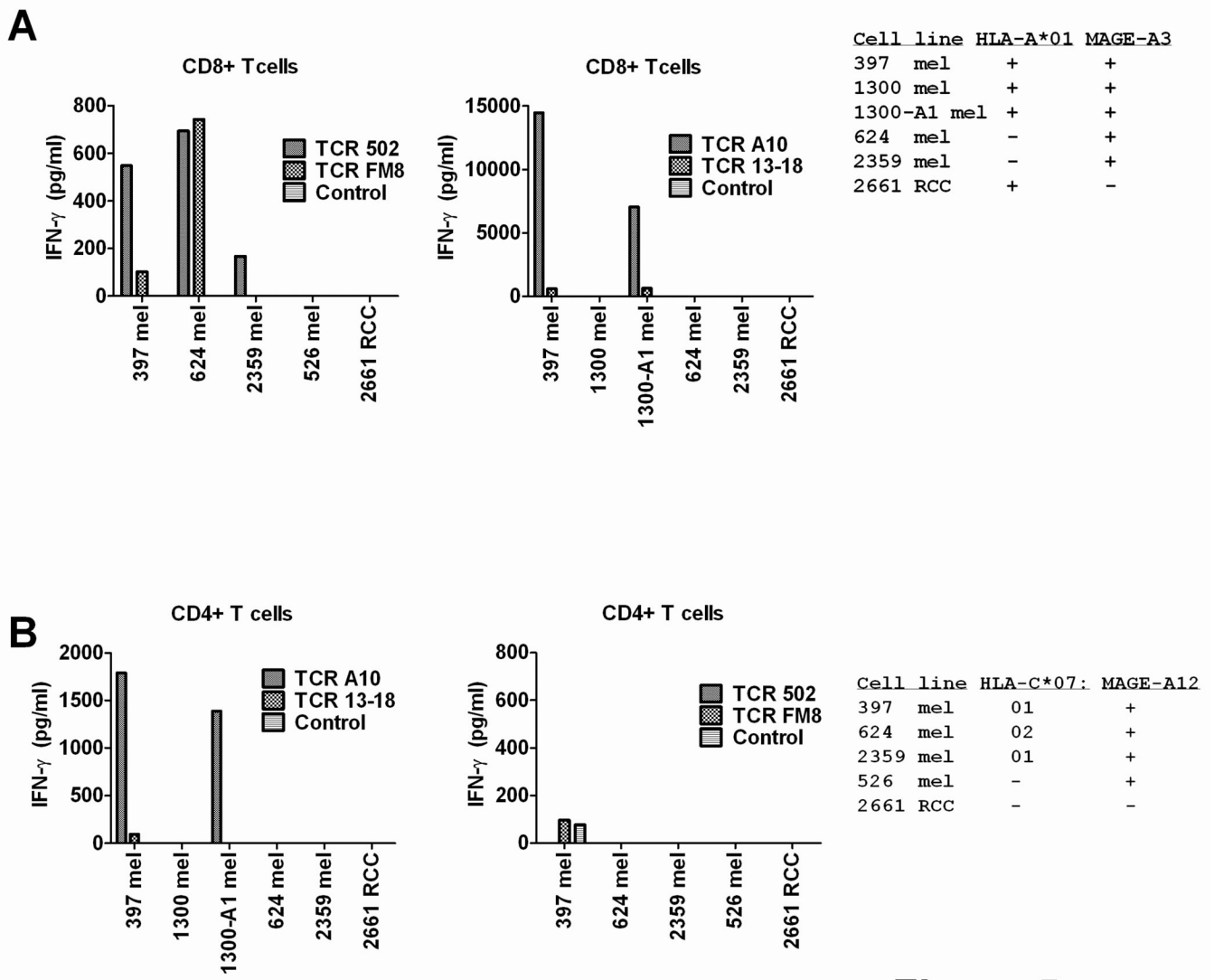
A

Cell line	HLA-A*01	MAGE-A3
397 mel	+	+
1300 mel	+	+
1300-A1 mel	+	+
624 mel	−	+
2359 mel	−	+
2661 RCC	+	−

B

Cell line	HLA-C*07:	MAGE-A12
397 mel	01	+
624 mel	02	+
2359 mel	01	+
526 mel	−	+
2661 RCC	−	−

Figure 5.
Response of TCR transduced CD8+ and CD4+ T cells to tumor targets. Populations of purified CD8+ and CD4+ T cells were incubated with tumor cells in an overnight co-culture and the release of IFN-γ was measured by ELISA. Representative results from one of two independent experiments are presented.

Table 1

Comparison of sequences of MAGE-A family members

MAGE family member	HLA restriction element	Epitope region
MAGE-A3	A*01	EVDPIGHLYIF
MAGE-A12	C*07	EVVRIGHLYIL
MAGE-A1	-	EADPTGHSYVL
MAGE-A2	-	EVVPISHLYIL
MAGE-A4	-	EVDPTSNTYTL
MAGE-A6	-	EVDPIGHVYIF
MAGE-A9	-	EVDPAGHSYIL
MAGE-A10	-	EVDPTGHSFVL