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Focal adhesion kinase as an immunotherapeutic target

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Abstract

Background—Focal adhesion kinase (FAK) is a ubiquitously expressed non-receptor tyrosine kinase involved in cancer progression and metastasis that is found overexpressed in a large number of tumors such as breast, colon, prostate, melanoma, head and neck, lung and ovary. Thus, FAK could be an attractive tumor associated antigen (TAA) for developing immunotherapy against a broad type of malignancies. In this study, we determined whether predicted T cell epitopes from FAK would be able to induce anti-tumor immune cellular responses.

Methods—To validate FAK as a TAA recognized by CD4 helper T lymphocytes (HTL), we have combined the use of predictive peptide/MHC class II binding algorithms with *in vitro* vaccination of CD4 T lymphocytes from healthy individuals and melanoma patients.

Results—Two synthetic peptides, FAK₁₄₃₋₁₅₇ and FAK₁₀₀₀₋₁₀₁₄, induced HTL responses that directly recognized FAK-expressing tumor cells and autologous dendritic cells pulsed with FAK-expressing tumor cell lysates in an HLA class II-restricted manner. Moreover, since the FAK peptides were recognized by melanoma patient's CD4 T cells, this is indicative that T cell precursors reactive with FAK already exist in peripheral blood of these patients.

Conclusions—Our results provide evidence that FAK functions as a TAA and describe peptide epitopes that may be used for designing T cell-based immunotherapy for FAK-expressing cancers, which could be used in combination with newly developed FAK inhibitors.

Keywords

Focal adhesion kinase; Immunotherapy; Tumor Antigens; Major histocompatibility complex class II; CD4 helper T lymphocytes; Peptide epitope

Introduction

Many of the tumor-associated antigens (TAA) that are recognized by the immune system (T lymphocytes, antibodies) can also be found on normal cells, but usually at much lower concentrations as compared to tumor cells. Identification of peptide epitopes of overexpressed TAA that are recognized by tumor-reactive CD8 cytotoxic T lymphocytes (CTL) and CD4 helper T lymphocytes (HTL) is necessary for developing T cell-based

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immunotherapies such as peptide vaccines or adoptive T cell therapy to treat and/or prevent relapsing tumors. Clinical responses, which have been observed in some patients undergoing immunotherapy, provide encouragement to continue to identify novel TAA for improving T cell-based tumor immunotherapy [22,23]. In particular, it would be significant to develop clinical immune interventions for a broad population of cancer patients regardless of their tumor type, by focusing in TAA that are widely expressed by many types of cancers [21].

Focal adhesion kinase (FAK) is a ubiquitously expressed non-receptor tyrosine protein kinase that provides signaling and scaffolding functions at sites of integrin adhesion, which has been linked to providing anti-apoptotic functions for migrating cells [8,13,16,28,30]. Although FAK expression is required for many normal cellular functions, it also plays a significant role in cell survival, migration, invasion and metastasis for cancer cells [18]. Several studies have indicated the contributions of FAK in cancer development, such as tumor growth, via signaling through RAS-MAP kinase [1,11,12], caspase 3 inhibition for survival [4,10,35] and JNK-matrixmetalloproteinase induction, which enhances invasion and metastasis [13]. Recently, using a mouse skin carcinogenesis model it was reported that FAK-dependent survival signaling is required for tumor formation and progression, indicating that this effect is associated with the anti-apoptotic function of FAK [19]. Although FAK itself has not been demonstrated to function as an oncogene, its overexpression has been reported in a broad range of human cancers including breast, colon, prostate, head and neck, lung, ovary and melanoma [2,5-7,9,17,27,33]. Most importantly, FAK overexpression is especially found in metastatic lesions and has been correlated with a poor prognosis [25,26,31].

In view of the above, we postulate that FAK would be an attractive target for developing anti-tumor immunotherapy. We describe here for the first time that FAK can function as a TAA since it is capable of being recognized by tumor-reactive T lymphocytes. Our studies resulted in the identification of two novel peptide epitopes from FAK that were capable of inducing CD4 T cell responses to FAK-expressing tumor cells. These results may be relevant for the future development of T-cell based immunotherapy that could be combined with newly designed FAK inhibitors enhancing the efficacy of anti-FAK cancer treatments.

Materials and Methods

Cell lines

Epstein-Barr virus (EBV)-transformed lymphoblastoid cells (EBV-LCL) were produced from peripheral blood mononuclear cells (PBMC) of HLA-typed volunteers using culture supernatant from the EBV-producing B95-8 cell line, obtained from the American Type Culture Collection (ATCC, Manassas, VA). Mouse fibroblasts cell lines (L-cells) transfected and expressing individual human MHC-II molecules were kindly provided by Dr. Robert W. Karr (Idera Pharmaceuticals, Essex, CT) and Dr. Takehiko Sasazuki (International Medical Center of Japan, Tokyo, Japan). The following tumor cell lines were obtained from the ATCC: LNCaP, PC3 and DU145 (prostate cancers); MCF7 and SK-BR-3 (breast cancers); SK-MEL-15 (melanoma); WiDr (colon cancer) and Raji (Burkitt's lymphoma). The prostate cancer cell line LAPC4 was provided by Dr. Charles Sawyers (University of California at Los Angeles, CA). The melanoma cell lines 624mel, 697mel and 888mel were provided by Dr. Steven Rosenberg (Surgery Branch, National Cancer Institute, NIH, Bethesda, MD). The HTLV-1 infected T cell lymphoma cell lines Su, Kan, Hut102 and Hir were supplied by the Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer (Tohoku University, Sendai, Japan). The HTLV-1 infected T cell lymphoma cell line OKM2T was purchased from Dainippon Sumitomo Pharma (Osaka, Japan). The myeloid leukemia cell line KT1 was kindly provided by Dr. Masaki Yasukawa (Ehime University, Ehime, Japan). All cell lines were maintained in tissue culture as recommended by the supplier.

Synthetic peptides

Potential HLA-DR-restricted CD4⁺ T-cell epitopes were selected from the amino acid sequence of the FAK protein using the peptide/MHC binding prediction algorithm tables derived for three HLA-DR alleles (*DRB1*0101*, *DRB1*0401*, and *DRB1*0701*) as described [32]. The predicted peptide epitopes were synthesized by solid phase organic chemistry and purified by high performance liquid chromatography (HPLC). The purity (>80%) and identity of peptides were assessed by HPLC and mass spectrometry, respectively. The following synthetic peptides were used throughout this work: FAK₁₄₃₋₁₅₇ (TLNFFYQQVKSDYML), FAK₁₀₀₀₋₁₀₁₄ (KMKLAQQYVMTSLQQ) and the Pan DR Epitope “PADRE” (aKXVAAWTLKAAa, where a is D-alanine, and X is L-cyclohexylalanine).

In vitro induction of antigen-specific HTL with synthetic peptides

The procedure utilized for the generation of FAK-reactive HTL lines using peptide-stimulated lymphocytes has been described in detail [15]. Briefly, dendritic cells (DC) were produced in tissue culture from purified CD14 monocytes (using antibody-coated magnetic microbeads from Miltenyi Biotech, Auburn, CA) that were cultured for 7 days at 37 °C in a humidified CO₂ (5%) incubator in the presence of 50 ng/ml GM-CSF and 1000 IU/ml IL-4. Peptide-pulsed DC (3 µg/ml for 2 hr at room temperature) were irradiated (4,200 rads) and co-cultured with autologous purified CD4 T cells (using antibody-coated magnetic microbeads from Miltenyi Biotech) in 96-round bottom well culture plates. One week later, the CD4 T cells were restimulated in individual microcultures with peptide-pulsed irradiated autologous PBMC and two days later, human rIL-2 was added at a final concentration of 10 IU/ml. One week later, the T-cells were tested for antigen reactivity using a cytokine-release assay as described below. Those microcultures exhibiting a significant response of cytokine-release to peptide (at least 2.5 fold over background) were expanded in 24 or 48-well plates by weekly restimulation with peptides and irradiated autologous PBMC. Complete culture medium for all procedures consisted of AIM-V medium (Invitrogen/GIBCO, Carlsbad CA) supplemented with 3% human male AB serum. All blood samples were obtained after the appropriate informed consent.

Measurement of antigen-specific responses with HTL lines

CD4 T cells (3×10^4 /well) were mixed with irradiated APC in the presence of various concentrations of antigen (peptides, tumor cell lysates), in 96-well culture plates. APC consisted of either autologous PBMC (1×10^5 /well), HLA-DR-expressing L-cells (3×10^4 /well), MHC typed EBV-LCL and T cell lymphoma (3×10^4 /well), autologous DC (5×10^3 /well) or prostate, melanoma and breast tumor cell lines (3×10^4 /well that were previously treated with IFN- γ at 500 units/ml for 48 hr to enhance MHC antigen expression). The expression of HLA-DR molecules on tumor cells was evaluated by flowcytometry using anti-HLA-DR (L243) monoclonal antibody (mAb) conjugated with fluorescein isothiocyanate. Tumor cell lysates were prepared by 3 freeze-thaw cycles of 1×10^8 tumor cells, resuspended in 1 ml of serum-free RPMI-1640 medium. Tumor cell lysates were used as a source of antigen at 5×10^5 cell equivalents per ml. Culture supernatants were collected after 48 hr for measuring antigen-induced lymphokine (IFN- γ or GM-CSF) production by the HTL ELISA kits (BD Pharmingen, San Diego, CA). To demonstrate antigen-specificity and MHC restriction, blocking of antigen-induced responses were assessed by adding anti-HLA-DR mAb L243 (IgG2a, prepared from supernatants of the hybridoma HB-55 obtained from the ATCC), or anti-HLA-A/B/C mAb W6/32 (IgG2a, ATCC) at 10 µg/ml throughout the 48 hr incubation-period. All ELISA determinations were carried out in triplicate and results correspond to the mean values with the standard deviation (SD) of the mean.

Western blot analyses

One million tumor cells were washed in PBS and lysed in NuPAGE LDS sample buffer (Invitrogen). The tumor cell lysate was subjected to electrophoresis in a 4% to 12% NuPAGE bis-Tris SDS-PAGE gel (Invitrogen) under reducing condition and then transferred to Immobilon-P (Millipore, Bedford, MA) membrane. The membrane was then blocked in PBS containing 0.01% Tween20 and 5% nonfat dry milk for 1h at room temperature and incubated with anti-FAK (H-1) mouse monoclonal antibody (1:200 in blocker; Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. After washing, the membrane was incubated with horseradish peroxidase-labeled sheep anti-mouse IgG and subjected to the enhanced chemiluminescence assay using the ECL detection system (Amersham, Buckinghamshire, UK).

Immunohistochemistry

Expression of FAK was determined by immunohistochemical analysis using formalin-fixed paraffin-embedded sections of tumors. Sections were deparaffinized and incubated with 1% hydrogen peroxidase in methanol for 30 min to block endogenous peroxidase activity. Then the sections were incubated at 4 °C overnight with mouse antihuman FAK antibody clone 4.47 (Upstate Biotechnology, Waltham, MA) at 1:200 dilution. The sections were washed in PBS and subsequently incubated with Envision Plus (Dako Cytoformation, Kyoto, Japan). The chromogenic reaction was performed using 3,3'-diaminobenzidine tetrahydrochlorine for 5 min. Counterstaining with Giemsa for 1 min was the final step.

Results

Selection of potential MHC class II restricted peptide-epitopes from FAK

The identification of the broadly degenerate (promiscuous) HLA class II-binding peptides will enhance their use as epitope-based vaccines in the general patient population. For predicting promiscuous MHC class II-binding peptides (i.e., capable of binding to more than one MHC class-II allele) from FAK, we used computer-based MHC peptide-binding algorithms [32], which are based on peptide binding motifs for 3 common HLA class II molecules, HLA-DR1, DR4 and DR7. In our past experience, this algorithm system proved to be effective for identifying HTL epitopes from numerous TAA [14]. Notably, some peptides predicted by the algorithm to serve as promiscuous epitopes (i.e. to bind to DR1, DR4 and DR7) were found to stimulate T cell responses that were restricted by MHC class II molecules other than HLA-DR1, DR4 and DR7, such as DR9, DR15, DR16, D52, DR53, DQ2 and DQ6. For the present studies, two peptide sequences, FAK₁₄₃₋₁₅₇ (TLNFFYQQVKSDYML) and FAK₁₀₀₀₋₁₀₁₄ (KMKLAQQYVMTSLQQ), were signaled out as potentially binding to the 3 MHC class II alleles (data not presented), suggesting that these peptides could serve as promiscuous CD4 T cell epitopes.

Induction of CD4 T cell responses to FAK epitopes

To elicit HTL responses against FAK, CD4 T cells purified from four healthy individuals (HLA-DR1/15, HLA-DR4/9, HLA-DR4/15 and HLA-DR9/14) were stimulated with autologous DC loaded with the individual FAK-derived peptides. A total of 96 microcultures containing CD4 T cells, DC and peptide were set up for each donor and 7 days later each individual microculture was restimulated with peptide-pulsed irradiated autologous PBMC as APC. After 2-3 restimulation cycles, the ability of the FAK peptides, FAK₁₄₃₋₁₅₇ and FAK₁₀₀₀₋₁₀₁₄ to induce HTL responses was examined by the production of lymphokine (IFN- γ or GM-CSF) when stimulated with peptide-pulsed autologous PBMC. Out of the 4 donors, the HLA-DR4/9 and DR9/14 donor's CD4 T cells responded to both peptides FAK₁₄₃₋₁₅₇ and FAK₁₀₀₀₋₁₀₁₄ and the DR4/15 donor's CD4 T cells reacted with peptide FAK₁₄₃₋₁₅₇ (data not

shown). However, the CD4 T cells from the HLA-DR1/15 individual did not react with either peptide (data not presented). Those microcultures that exhibited responses to peptide stimulation were cloned by limiting dilution and expanded to assess their antigen specificity, MHC restriction and anti-tumor reactivity. As shown in Figure 1, three peptide FAK₁₄₃₋₁₅₇-reactive clones (143-1 from DR4/9 donor, 143-2 from DR9/14 donor and 143-3 from DR4/15 donor) and four peptide FAK₁₀₀₀₋₁₀₁₄-reactive clones (1000-1, 1000-2 from DR4/9 donor and 1000-3, 1000-4 from DR9/14 donor) were isolated and shown to respond to antigen in a specific manner. Antigen titration curves revealed lymphokine production to peptide-pulsed autologous PBMC occurring in a peptide dose-dependent manner, but the overall avidity of the T cells for antigen, as determined by the amount of peptide required to attain the maximal response varied from clone to clone (Fig. 1).

MHC class II restriction analyses of FAK-reactive CD4 T cells

The FAK-reactive HTL clones were analyzed for their MHC class II-restriction pattern first using mAbs specific for HLA-DR molecules (L243) and HLA-A, B, C molecules (W6/32 as negative control) to block antigen presentation and later utilizing mouse fibroblast lines (L cells) expressing individual HLA-DR molecules, which were used as APC to assess the capacity of distinct MHC class II molecules to present peptide. As shown in Figure 2, recognition of peptide-pulsed autologous PBMC by all the CD4 T cell clones was inhibited by the addition of anti-HLA-DR mAb but not by the anti-MHC class I mAb, indicating that the presentation of peptides FAK₁₄₃₋₁₅₇ and FAK₁₀₀₀₋₁₀₁₄ to these HTL clones is via HLA-DR molecules and not other MHC class II molecules such as HLA-DP or DQ. Subsequently, experiments using a panel of MHC class II expressing L-cells as APC revealed that FAK₁₄₃₋₁₅₇-reactive clones 143-1, 143-2 and 143-3 recognized antigen in the context of HLA-DR9, DR53 and DR4, respectively (Fig. 3). In the case of the FAK₁₀₀₀₋₁₀₁₄, clone 1000-1 recognized antigen in the context of HLA-DR9 while clones 1000-2, 1000-3 and 1000-4 did so in the context of HLA-DR53. These results indicate that peptides FAK₁₄₃₋₁₅₇ and FAK₁₀₀₀₋₁₀₁₄ behave as classic promiscuous CD4 T helper epitopes because both can be presented to T cells by more than one MHC class II allele.

CD4 T cell epitopes FAK₁₄₃₋₁₅₇ and FAK₁₀₀₀₋₁₀₁₄ are naturally processed and presented by FAK-expressing tumor cells

In some instances we have observed that CD4 T cells can directly recognize MHC class II-expressing tumor cells indicating that some epitopes can be naturally processed and by the tumor cells. Thus, we first proceeded to study the expression of FAK protein and MHC class II molecules in various tumor cell lines that could be used to assess direct antigen presentation to the CD4 T cell clones. The results presented in Figure 4 show that the FAK protein is expressed by various tumors including prostate (LNCaP, PC3, DU145, LAPC4), melanomas (624mel, 697mel, 888mel, SK-MEL-15), breast (SK-BR-3, MCF7) colon (WiDr), HTLV-1⁺ T-cell lymphomas (Su, Kan, Hut102), a myeloid leukemia (KT1) and a Burkitt's lymphoma (Raji). Since some of these tumor cell lines express cell surface MHC class II molecules (Fig. 4 lower panels), and more importantly they have the corresponding MHC class II restriction alleles for the CD4 T cell clones, we assessed the capacity of some of the FAK expressing tumor cells to directly stimulate the FAK-reactive T cells. The data presented in Figure 5A shows that the DR9-restricted, FAK₁₄₃₋₁₅₇-reactive clone 143-1 recognized the FAK⁺, DR9-expressing T-cell lymphomas, Su and Kan but not the FAK-negative DR9-expressing T-cell lymphoma Hir. In turn, the HLA-DR53-restricted, FAK₁₄₃₋₁₅₇-reactive clone 143-2 directly recognized several FAK⁺ DR53-expressing tumor cell lines such as PC3, 697mel, SK-MEL-15 and WiDr (Fig. 5B), but were unable to respond to FAK negative, DR53-expressing cell lines (Hir and EBV-Wa). The FAK₁₀₀₀₋₁₀₁₄-reactive HLA-DR4-restricted clone 1000-1 was able to recognize FAK⁺, DR4-expressing SK-MEL-15 and WiDr tumors but not FAK-negative, DR4-expressing EBV-Wa cells or FAK-positive, DR4-negative PC3 (Fig. 5C). Similarly, we

observed that the HLA-DR53-restricted, FAK₁₀₀₀₋₁₀₁₄-reactive clones 1000-2, 1000-3 and 1000-4 were all capable of recognizing antigen directly on DR53-expressing, FAK⁺ tumors (PC3, 697mel and SK-MEL-15) but did not react with the DR53-expressing, FAK-negative EBV-Wa cell line (Fig. 5D). It should be noted that in all cases the direct recognition of FAK-expressing tumor cells by the T cell clones was inhibited by the addition of anti-HLA-DR mAb, indicating that peptide-epitopes that were presented on the surface of tumor cells were processed and presented via the MHC class II pathway. Taken together, the overall results demonstrate that epitopes FAK₁₄₃₋₁₅₇ and FAK₁₀₀₀₋₁₀₁₄ are naturally processed and presented by a variety of human tumor cells of different histological origin such as prostate, melanoma, colon and T-cell lymphomas.

Recognition of naturally processed FAK protein presented by autologous DC

The above results indicate that FAK-reactive CD4 T cells can recognize antigen directly on MHC class II-expressing FAK-positive tumor cells. However, since many tumor cells express low levels of cell surface MHC class II molecules, we considered necessary to assess whether professional APC such as DC that capture dead tumor cells, could subsequently process the FAK antigen and present the peptide-epitopes to HTL. Thus, we prepared freeze-thaw cell lysates from FAK-positive tumors (LNCaP, 697mel, Raji and KT1) and fed them to DC. The FAK-reactive HTL were then tested for their ability to recognize the naturally processed epitopes presented by the autologous DC that had ingested the tumor cell lysates. As shown in Figure 6, the DR53-restricted, FAK₁₀₀₀₋₁₀₁₄-reactive HTL clones 1000-2 and 1000-4 were both efficient in recognizing naturally processed epitopes presented by DC derived from FAK⁺ tumor cell lysates but not FAK-negative Hir T-cell lymphoma cell lysate. These responses were inhibited by anti-HLA-DR mAb, confirming that the peptide epitopes were presented in the context of MHC class II molecules. In contrast, tumor cell lysate-pulsed DC were unable to stimulate any of the FAK₁₄₃₋₁₅₇-reactive HTL clones (data not shown), although as previously shown these HTL were fully capable of directly recognizing FAK⁺ tumor cells (Fig. 5) and peptide-pulsed conventional APC (Figs. 1-3) including peptide-pulsed DC (data not shown). Overall, these results indicate that FAK⁺ tumor cells process the FAK₁₄₃₋₁₅₇ epitope in a different manner as compared to professional APC.

Recognition of peptides FAK₁₄₃₋₁₅₇ and FAK₁₀₀₀₋₁₀₁₄ by lymphocytes from melanoma patients

Lastly, we considered important to examine whether T cells from cancer patients (melanoma) would be able to respond to the newly identified FAK₁₄₃₋₁₅₇ and FAK₁₀₀₀₋₁₀₁₄ epitopes. Peripheral blood lymphocytes from 3 melanoma patients with tumors expressing FAK (Fig. 7, upper panels) were studied for their ability to produce lymphokines (GM-CSF and IFN- γ) when stimulated *in vitro* with various synthetic peptides. The data presented in Figure 7 (lower panels) indicates that both FAK₁₄₃₋₁₅₇ and FAK₁₀₀₀₋₁₀₁₄ peptides and a positive control (PADRE) peptide (3) were quite effective in inducing cytokine production in all 3 patients. Because only a small volume of blood was available from these patients, we were unable to establish long-term T cell lines and to perform HLA typing, limiting the depth of these studies. Nevertheless, these results indicate peptides FAK₁₄₃₋₁₅₇ and FAK₁₀₀₀₋₁₀₁₄ were readily recognized by melanoma patient's lymphocytes, suggesting that T-cell precursors reactive with these FAK epitopes exist in their peripheral blood.

Discussion

Immune-based therapy based on the induction of T lymphocyte responses against specific TAA has become an attractive alternative therapeutic approach to treat various types of malignancies. Therefore, the identification of clinically relevant TAA and their corresponding peptide epitopes remains a priority for the design of effective vaccination strategies or adoptive T-cell

therapies. Although a large number of TAA have already been identified for developing immunotherapies, some researchers believe that it would be preferable to focus on those TAA that are broadly expressed throughout many tumor types (i.e., “universal antigens”), especially those that participate in the malignant function of the transformed cell. The non-receptor tyrosine kinase FAK fits well in this category of TAA since it is found overexpressed in various tumor types and is involved in adhesion, migration, invasion and survival of the tumor cells. As with the majority of universal TAA, message and protein levels of FAK were shown to be absent or in low amounts in normal tissues and benign neoplasms, while these levels are found upregulated in invasive and metastatic tumors [24,25,34]. Our results suggest that T cell responses against epitopes FAK₁₄₃₋₁₅₇ and FAK₁₀₀₀₋₁₀₁₄ would not affect many normal tissues since in the absence of peptide or tumor cell lysate, all the normal APC such as PBMC, DC and mouse fibroblast L-cells (the sequences of the 2 epitopes are conserved between humans and mice), did not stimulate the antigen specific HTL. However, we cannot totally discount the possibility that a specific tissue could harbor cells that under normal conditions may overexpress FAK and MHC class II molecules. Thus, we believe that with the data available so far, no significant concerns should exist of inducing severe autoimmune pathology when utilizing FAK as a TAA. Nevertheless, it could prove to be useful to assess the levels of FAK in normal tissues before embarking in clinical studies using FAK as a target for immunotherapy.

The goal of the present study was to assess whether FAK could function as a TAA for T lymphocytes, specifically for MHC class II-restricted CD4 T cells. Using a peptide/MHC-II binding prediction algorithm we centered our study in determining whether 2 predicted peptides, FAK₁₄₃₋₁₅₇ and FAK₁₀₀₀₋₁₀₁₄, would be capable of eliciting anti-tumor T cell responses using an *in vitro* immunization protocol. The results show that indeed, both peptides were efficient in activating and expanding CD4 T cells obtained from normal individuals. Most significant was the demonstration that the CD4 T cells generated by peptide stimulation were capable of recognizing naturally processed FAK antigen either directly on MHC-II expressing FAK-positive cells or indirectly by autologous DC that were fed tumor cell lysates. In addition, both of the FAK CD4 T cell epitopes were able to stimulate T cell responses in some melanoma patients bearing FAK-positive tumors. It remains to be determined whether cancer patients suffering from other type of malignancies besides melanoma would be able to mount CD4 T cell responses against FAK-derived epitopes. Nevertheless, our results indicate that T cell precursors capable of recognizing FAK MHC-II restricted epitopes exist both in normal individuals and some cancer patients. At present time we do not know whether the induction of CD4 T cell responses to FAK via a therapeutic vaccine would translate to a clinical benefit against tumors that overexpress FAK. Nevertheless, we believe that the simultaneous induction of FAK-specific CD4 and CD8 T cell responses would be more effective in achieving anti-tumor effects than the separate induction of CD4, or CD8 T cell responses. Thus, some efforts will be required to identify MHC-I restricted CD8 T cell epitopes from FAK before this approach can be taken into the clinic. Finally, the recent development of FAK inhibitors as anti-cancer therapeutics [20,29] opens the interesting opportunity of a combined chemotherapy/immunological approach for treating FAK-expressing tumors.

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Abbreviations

APC, Antigen presenting cell(s); DC, Dendritic cell(s); FAK, Focal adhesion kinase; HTL, Helper T lymphocyte(s); TAA, Tumor associated antigen(s).

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Fig. 1.

Induction of CD4 T cell responses using predicted peptide epitopes derived from FAK. *A, B*, HTL clones induced with peptide FAK₁₄₃₋₁₅₇ (143-1 from DR4/9 donor, 143-2 from DR9/14 donor and 143-3 from DR4/15 donor) were tested for their capacity to recognize autologous PBMC as APC in the presence of various concentrations of peptide. *C*, results with HTL clones obtained with peptide FAK₁₀₀₀₋₁₀₁₄ (1000-1 and 1000-2 from the DR4/9 donor, and 1000-3 and 1000-4 from the DR9/14 donor). Points, means of triplicate determinations; bars, SD. Points without bars had SD < 10 % the value of the mean. Results are representative of two experiments that were done with the same samples.

Fig. 2.

The presentation of FAK peptides to CD4 T cells is in the context of HLA-DR. Monoclonal Abs specific for HLA-DR (L243 used at 10 μ g/ml) but not for HLA-A, -B, -C (W6/32) inhibited the lymphokine production from the FAK₁₄₃₋₁₅₇-specific HTL clones (A and B) and FAK₁₀₀₀₋₁₀₁₄-specific HTL clones (C) stimulated with peptide-pulsed autologous PBMC. Columns, means of triplicate determinations; bars, SD. Columns without bars had SD < 10 % the values of the mean. Results are representative of two similar experiments that were done under the same conditions.

Fig. 3.

Definition of restricting MHC class II alleles of FAK-specific HTL clones. Lymphokine production of the FAK₁₄₃₋₁₅₇-reactive HTL clones (A and B) and FAK₁₀₀₀₋₁₀₁₄-reactive HTL clones (C) was evaluated using L-cells transfected with individual HLA-DRs used as APC to define the restricting HLA class II elements. Columns, means of triplicate determinations; bars, SD. Columns without bars had SD < 10 % the values of the mean. Results are representative of at least two experiments.

Fig. 4. Expression of FAK protein and cell surface MHC class II molecules by various human tumor cell lines. Upper panels: Western blot analysis was done using a FAK-specific mAb as described in Materials and Methods. The FAK protein has a mass of approximately 125 kDa. Lower panels: Expression of HLA-DR molecules on tumor cells evaluated by flowcytometry using anti-HLA-DR mAb L243 conjugated with fluorescein isothiocyanate (thick line open histograms). Staining with the isotype-negative control (filled histograms).

Fig. 5.

Direct recognition of FAK-expressing tumor cells by FAK-reactive HTL clones. **A**, HLA-DR9-restricted, FAK₁₄₃₋₁₅₇-specific HTL clone 143-1 was tested for its capacity to recognize antigen directly on HLA-DR9+, FAK+ tumor cells (Su and Kan) and DR9+, FAK-negative Hir cells **B**, HLA-DR53-restricted, FAK₁₄₃₋₁₅₇-specific HTL clone 143-2 was tested for its capacity to recognize antigen directly on HLA-DR53+, FAK+ tumor cells (PC3, 697mel, SK-MEL-15 and WiDr), DR53+, FAK-negative Hir and EBV-Wa. **C**, HLA-DR4-restricted, FAK₁₀₀₀₋₁₀₁₄-specific HTL clone 1000-1 was tested for its capacity to recognize antigen directly on HLA-DR4+, FAK+ tumor cells (SK-MEL-15 and WiDr), DR4+, FAK- EBV-Wa and DR4-, FAK+ PC3. Similar determinations were done with the HLA-DR53-restricted, FAK₁₀₀₀₋₁₀₁₄-reactive HTL clones 1000-2, 1000-3 and 1000-4 (**D**) using HLA-DR53+, FAK+ tumor cells (PC3, 697mel and SK-MEL-15) and DR53+, FAK-EBV-Wa. All FAK-reactive HTL responses against tumor cells were inhibited by anti-HLA-DR mAb L243 (used at 10 μ g/ml). Columns, means of triplicate determinations; bars, SD. Columns without bars had SD < 10 % the values of the mean. Results are representative of two separate experiments.

Fig. 6. FAK-reactive HTL recognize naturally processed exogenous antigen presented by autologous DC. FAK₁₀₀₀₋₁₀₁₄-reactive HTL clones 1000-2 (A) and 1000-4 (B) were tested for reactivity against FAK+ tumor cell (LNCaP, 697mel, Raji and KT1) lysates (Lys) and FAK-negative Hir cell lysate when presented by autologous DC. The capacity of anti-HLA-DR mAb L243 to inhibit these responses was assessed to demonstrate that T cell reactivity was through TCR/MHC interactions. Columns, means of triplicate determinations; bars, SD. Columns without bars had SD < 10 % the values of the mean. Results are representative of at 2 separate experiments.

Fig. 7.

Assessment of T cell responses to the FAK₁₄₃₋₁₅₇ and FAK₁₀₀₀₋₁₀₁₄ epitopes in melanoma patients. PBMC from melanoma patients whose surgical samples demonstrate FAK protein expression (A-C, *upper panels*) were stimulated with peptides and the T-cell responses (production of IFN- γ and GM-CSF, A-C, *lower panels*) were measured as described in Materials and Methods. Columns, means of triplicate determinations; bars, SD. Columns without bars had SD < 10 % the values of the mean. Results are representative of at least two separate experiments.