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Alpha-fetoprotein specific CD4 and CD8 T cell responses in patients with hepatocellular carcinoma

Shahriar Behboudi, Stephen P Pereira

Shahriar Behboudi, Stephen P Pereira, UCL Institute of Hepatology, University College London, Royal Free Hospitals, London NW3 2QG, United Kingdom
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Correspondence to: Shahriar Behboudi, PhD, UCL Institute of Hepatology, University College London, 69-75 Chenies Mews, London WC1E 6HX, United Kingdom. s.behboudi@ucl.ac.uk
Telephone: +44-20-76796515 Fax: +44-20-73800405
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Abstract

The presence of CD8 T cell responses to tumor associated antigens have been reported in patients with different malignancies. However, there is very little information on a comparable CD8 and CD4 T cell response to a tumor antigen in liver cancer patients. Here, we re-examine the kinetic and the pattern of T helper 1 and cytotoxic T lymphocyte responses to alpha-fetoprotein (AFP), a tumor rejection antigen in hepatocellular carcinoma (HCC). Then, we discuss the possibility of using AFP-based immunotherapy in combination with necrotizing treatments in HCC patients.

Key words: Hepatocellular carcinoma; Alpha-fetoprotein; Cell-mediated immunity; Immunotherapy

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide and a major health problem in parts of Asia and Africa. The majority of cases of HCC arise in cirrhotic livers with hepatitis B being the main cause of nodules developing in the cirrhotic liver with malignant transformation resulting in HCC. Survival of untreated patients is poor and surgical resection provides the only chance of cure. However, surgery is not suitable for majority of patients in whom the tumor is metastasized and/or liver function is seriously undermined. For the majority of the HCC patients, non-surgical treatments such as transarterial chemoembolization and radiofrequency ablation are the only option[1]. These nonsurgical treatments have been shown to reduce tumor burden and improve survival rate but tumor relapse is common and thus more effective treatments are required to control tumor growth[2]. Early diagnosis and the development of novel systemic therapies such as immunotherapeutic strategies may be very important. It has been demonstrated that the immune system is able to induce responses against tumors and these responses can be enhanced using a number of strategies. T cells or T lymphocytes are a group of immune system cells that play a central role in cell-mediated immunity. Cytotoxic T cells (CD8 T cells) and helper T cells (CD4 T cells) recognize tumor associated antigens presented on MHC class Ⅰ or Ⅱ of antigen presenting cells via their T cell receptors. The activated T cells develop into effector and memory T

cells that recognize and lyse tumor cells. Molecular and immunological approaches have been applied to identify HCC associated antigens. HCC over-express several tumor associated antigens[3]. Some of these antigens such as MAGE, Glypican-3 and NY-ESO-1 are also expressed by many other types of cancer cells[4,5]. Among these antigens, alpha-fetoprotein (AFP) is shown to be specific to HCC and testicular carcinoma. AFP is a glycoprotein with a molecular weight of around 70 kDa which is produced in the endodermal cells of the yolk sac and fetal liver. The synthesis of AFP decreases dramatically after birth and only trace amounts are expressed in the adult liver. However, expression of the AFP gene is reactivated in adults during liver regeneration and hepatocarcinogenesis[6] with the majority of HCC patients showing an increase of AFP in serum. The measurement of serum AFP plays an important role in the diagnosis of HCC and monitoring responses to the treatment[6]. In some cases, over-expression of AFP can be detected in HCC cells even when serum AFP levels are normal[7].

The induction of anti-AFP cell-mediated immune responses has been demonstrated to control tumor growth in animal models. In one study, anti-AFP cytotoxic T lymphocyte (CTL) response was induced and a significant survival benefit was observed in mice immunized with mAFP expression vector DNA while no hepatocyte damage was detectable despite low-level endogenous hepatic mAFP expression, showing that AFP is a tumor rejection antigen[8]. In humans, it has been shown that B and T cells can recognize peptide epitopes within the AFP sequence and develop into effector and/or regulatory lymphocytes[9-17]. Here, we discuss naturally occurring CD8 and CD4 T cell responses to AFP in different groups of HCC patients and discuss the possibility of combining trans-arterial chemoembolization (TACE)/trans-arterial embolisation (TAE) treatment with AFP-based immunotherapy.

AFP-SPECIFIC CD8 T CELL RESPONSES

CD8 T cells recognize AFP derived peptide epitopes in the context of MHC class I molecules and develop into cytotoxic T cells with the ability to recognize and kill tumor cells. An existing immunological paradigm is that high concentrations of soluble protein contribute to the maintenance of peripheral tolerance/ignorance to self protein. However, that is not the case as many AFP-specific CD8 T cell clones are not deleted during ontogeny and AFP derived epitopes are recognized by both murine and human T cells. AFP-derived peptides with high probability to bind MHC class I (HLA-A2) were synthesized and tested *in vitro*. T cell clones recognizing several AFP-derived peptides were identified. Initially, four peptides were identified and termed "immunodominant" based on their binding efficiency to MHC class I (HLA-A2) and the ability to stimulate IFN-γ production by CD8 T cells from healthy donors. These peptide epitopes were also found to be immunogenic and immuno-dominant in HLA-A2 transgenic mice[18]. Later, five other AFP-derived peptides containing HLA-A2402 binding motifs were also identified. CD8 T cells recognized these epitopes presented on HLA-A2402 positive hepatoma cells and were developed into cytotoxic T cells with the ability to lyse tumor cells and produce IFN-γ[13]. Although several HLA-A2 and HLA-24 restricted CD8 T cell epitopes have been identified and some are classified as immuno-dominant and some as sub-dominant[11,13], it has been recently suggested that a high frequency of AFP-specific IFN-γ producing CD8 T cells (CTL) are directed against different epitopes spreading over the entire AFP sequence with no single immuno-dominant epitope[19]. Our recent data support these findings and demonstrate that there is no immunodominant CD8 T cell epitope within the AFP sequence[20]. Another important finding is that AFP-specific CD8 T cells can be detected in patients with HCC, patients with non-HCC liver diseases as well as healthy donors[19,21,22]. This is in contrast with the results demonstrating the absence of anti-AFP CD4 T cell responses in healthy donors or patients with non-HCC liver diseases[14,16,20]. The presence of CD8 T cells recognizing AFP, Glypican-3, NY-ESO-1 and MAGE-1 have been confirmed in healthy donors[10,19,23,24]. Although AFP-CD8 T cell responses are detected in non-HCC patients as well as HCC patients, it has been suggested that this response is stronger in HCC patients[19]. We were unable to confirm these findings and no significant differences were observed in the frequencies of anti-AFP CD8 T cell responses in healthy donors and patients with HCC and the same percentages of responders were observed in HCC patients and non-HCC patients[20]. The results from several studies including our recent report support the fact that the detection of AFP-specific CD8 T cells does not correlate with elevation of serum AFP, vascular invasion, liver function and the type of viral infection[19-21,25]. Similarly, no association was found between the levels of serum AFP and Okuda stage in HCC patients and the presence of CD8 T cell responses to non-AFP HCC associated antigens such as NY-ESO-1[26]. However, for the first time we have demonstrated that there is an association between the stage of liver cirrhosis and the presence of anti-AFP CD8 T cell responses. Anti AFP-CD8 T cell responses were observed in 17% of HCC patients with Child-Pugh A score while this response was detected in 46% of HCC patients with Child-Pugh B or C[20], demonstrating that anti-AFP CD8 T cell responses are expanded as liver cirrhosis progresses. Another interesting finding is that the frequency of AFP-specific CD8 T cells in the liver of HCC patients is no higher than that in peripheral blood[19]. This is an unusual finding, as tumor specific CD8 T cells are generally enriched in the liver[24]. It is possible that liver infiltrating AFP-specific CD8 T cells undergo apoptosis or stop responding to peptide stimulation due to exhaustion or expression of inhibitory molecules such as PD-1. This negative regulation by PD-1 on NY-ESO-1 specific CD8 T cell responses has been demonstrated in patients

with ovarian cancer[27] and further studies are required to establish its effects in patients with HCC. More sensitive assays for the detection of antigen specific CD8 T cells such as tetramer staining of AFP-specific CD8 T cells in liver and peripheral blood are required to confirm these findings. In a study performed in HLA-A24+ HCC patients, it was demonstrated that HCC treatments such as radiofrequency ablation (RF) or TACE can augment the frequency of circulating AFP-specific (*ex vivo*) but not viral-specific, CD8 T cells in some HCC patients[13]. This may suggest that tumor burden may suppress the expansion of anti-AFP CD8 T cell responses in HCC patients or that tumor necrosis stimulates the expansion of anti-tumor immune responses.

AFP-SPECIFIC CD4 T CELL RESPONSES

It has been shown that CD4 T cells play a crucial role in the control of tumor growth in both animal models and cancer patients. It is believed that CD4 T cells provide help required for the induction of CD8 T cell responses with the ability to lyse tumor cells in a MHC class Ⅰ, Fas ligand and perforin dependent manner. T helper (Th) 1 cells can also directly eradicate tumor cells without any significant involvement of CD8 T cells[28]. This is also confirmed in experiments involving adaptive transfer of IFN-γ producing CD4 T cells recognizing tumor antigens. The adaptive transfer of Th1 cells but not CTLs provided protection against various transplanted or endogenous tumors in animal models[29,30], suggesting that Th1 cells play a more important role in tumor immunity than was initially postulated. This is also shown in a cancer patient where adaptive transfer of a Th1 clone recognizing NY-ESO-1 antigen provided sustained clinical remission[31]. Therefore, it is crucial to explore the concept of targeting HCC-specific Th1 cells in anti-HCC immunotherapy.

We have extensively studied the magnitude and characteristics of circulating AFP-specific CD4+ T cell responses in HCC patients[14-16,20]. In these studies, several HLA-DR restricted CD4 T cell epitopes within the AFP sequence have been identified. In contrast to CD8 T cell responses, an immuno-dominant epitope is established for CD4 T cells and it is shown that more than 20% of HCC patients have a detectable response to this immuno-dominant epitope[14]. Th1 responses to the immunodominant epitopes were only observed in HCC patients and no response was detected in patients with non-HCC liver disease or healthy controls[14,16,20]. However, this does not exclude the possibility that Th1 responses to other yet unknown epitopes could be detected in patients with non-HCC liver disease or healthy controls. The presence of anti-AFP IgG in the serum of patients with non-HCC liver disease[17] suggests that AFP-specific CD4 T cell responses may be present in this group of patients. This notion is also supported by results showing that CD4 T cells from healthy donors respond to protein (AFP)-pulsed dendritic cells by producing IFN-γ[21].

Anti-AFP CD4 T cell response are more likely to be present in patients with early stage disease (Okuda stage Ⅰ) and low or moderately elevated serum AFP[14]. It is still unclear why AFP-specific CD8 T cell responses are detected in all groups of HCC patients (early and late stage cancer) and there is no association with the levels of serum AFP levels. We have recently performed a parallel analysis of AFP-specific CD4 and CD8 T cell responses in the same group of HCC patients and healthy donors. The results confirm this trend and demonstrated that anti-AFP Th1 response is detectable in 58% of HCC patients with Okuda stage Ⅰ tumors and 15.8% of patients with Okuda stage Ⅱ or Ⅲ tumors[20]. When the patients were classified based on their liver function, anti-AFP Th1 response was observed in 44% of HCC patients with a Child-Pugh A score (early stage of cirrhosis) whereas this response was detected in only 15% with a B or C score (late-stage cirrhosis). These results suggest that anti-AFP Th1 responses are more likely to be present in patients who are in an early stage of disease (for both tumor stage and liver cirrhosis). This indicates that there is a difference in the activation of anti-AFP CD4 *vs* CD8 T cells in HCC patients, with a CD4 T-cell response expanding in early stages of disease usually associated with low concentrations of serum AFP and with exhaustion of this response in later stages of disease in which there is a high concentration of serum AFP. This is in accordance with our earlier reports showing that high concentrations of AFP suppress immune cell function *in vitro*[32] and CD4 T cells isolated from HCC patients with high concentrations of serum AFP are impaired[14]. It seems that anti-AFP CD4 T cell response is impaired or exhausted in late stage HCC patients and any effective immunotherapy should be combined with treatment strategies that restore the function of these cells. It is possible that CD4 T cells are more susceptible to immuno-regulatory effects of tumor cells than that by CD8 T cells. In this case, the removal of tumor cells, the immuno-regulatory molecules or cells induced by tumor cells should improve the function of anti-tumor Th1 cells. In fact, we have shown that tumor necrotizing treatments such as TACE/TAE that reduce tumor burden improve the function of AFP-specific CD4 T cells[16]. Different immune-regulatory mechanisms have been reported in patients with HCC[15,32-34]. For example, an expansion of $CD4^+CD25^+$ regulatory T cells in the peripheral blood and tumors tissues of patients with HCC has been reported[33,35,36] and this expansion has an inverse correlation with the recurrence-free survival[37]. A number of preclinical murine studies suggest that the depletion of regulatory T cells augments the effects of immune-based therapies such as anti-tumor vaccines[38]. The lack of a specific marker for the detection of $CD4^+$ regulatory T cells makes their *in vivo* depletion in patients difficult. However, low-dose cyclophosphamide treatment has been shown to deplete $CD4^+CD25^+$ regulatory T cells in a murine tumor model[39]. In a recent report, the effect of a low-dose cyclophosphamide treatment on the frequency and function of regulatory T cells in patients with advanced HCC were analyzed. A systemic treatment of HCC patients with low-dose cyclophosphamide decreased

the frequency and suppressor function of circulating $CD4^+CD25^+Foxp^+$ regulatory T cells in peripheral blood and unmasked anti-AFP T cell responses[40]. The results suggest that this procedure could be used in combination with immunotherapeutic approaches in HCC.

In a clinical trial, when both CD4 and CD8 T cells were targeted by administration of tumor lysate-pulsed dendritic cells in HCC patients, a partial clinical response was observed in one out of 35 HCC patients[41]. In this clinical trial[41], the induction or expansion of a pre-existing AFP-specific CD4 T cell response was not analyzed. However, we believe that this vaccination strategy can induce or activate both AFP-specific T helper and cytotoxic T cell responses required for the generation of potent anti-tumor immune responses. It is possible but not proven that tumor lysate-pulsed dendritic cells can also activate the expansion of regulatory T cells and this can diminish the effectiveness of this immunotherapy strategy[42]. In fact, it has been suggested that transforming growth factor-beta1 producing CD4 T cells could be induced by the AFP-derived peptide epitope[15] and this may hamper anti-tumor immunity.

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