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IMMUNOTHERAPY OF HUMAN PAPILLOMAVIRUS-ASSOCIATED MALIGNANCIES AND THE CHALLENGES POSED BY T-CELL TOLERANCE

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TABLE OF CONTENTS

1. ABSTRACT

Human papillomaviruses are associated with a broad range of carcinomas, including cervical cancer. Although the delivery of immunogenic tumor-associated antigens represents a promising approach in the treatment of these malignancies, the imposition of T cell tolerance poses a significant challenge in this endeavor. The purpose of this review is to discuss T cell tolerance and the role of T cell costimulation in the immunotherapy of HPV-associated malignancies.

2. INTRODUCTION

The neoplastic transformation of a cell, by most accounts, may be attributed to a multistep process involving many rounds of genetic mutation followed by natural selection within the host. Therefore, any physical, chemical, or biological conditions, which increase the rate of cellular proliferation or prevent a damaged cell from undergoing apoptosis may promote the neoplastic transformation of the cell. Exposure to chemical carcinogens and UV irradiation have long been associated with human malignancies; however, the last decade has witnessed a growing awareness of the link between infectious agents and human cancers (1), leading some to estimate that a significant portion of human cancers worldwide may be associated with viral infections (2, 3), including hepatitis B virus (hepatocellular carcinoma), Epstein Barr virus (Burkitt's lymphoma and nasopharyngeal carcinoma), hepatitis C virus (immunoblastic lymphoma), human T cell leukemia virus (adult T-cell leukemias and lymphomas), HHV-8 (Kaposi's sarcoma, Castleman's disease, and body cavity lymphomas), HHV-6 (non-Hodgkin's lymphoma) and human papillomaviruses.

Human papillomaviruses (family *papovaviridae*) are epitheliotropic, non-lytic, double-stranded circular DNA viruses, producing hyperkeratotic lesions with little to no inflammation. Infectious virions are thought to be shed from the epithelial surface within desquamating keratinocytes. The HPV genome may be divided into both early (E) genes, encoding proteins involved in DNA

replication, transcription and cellular transformation, and late (L) genes, which encode the viral capsid proteins, L1 and L2. HPV replication is exquisitely linked to the differentiation state of the keratinocyte. HPV particles are thought to infect basal keratinocytes coincident with local damage. Although viral gene expression is virtually undetectable in the basal layer, the early genes (including E1, E2, E5, E6, and E7) are transcribed as the keratinocyte differentiates and migrates from the basal layer, eventually to be exfoliated. An overwhelming body of evidence, too extensive to enumerate here, has implicated the E6 and E7 gene products as the major transforming proteins. This may be attributed to the ability of these viral oncogenes to associate with and functionally neutralize the tumor suppressors p53 and Rb (4-6).

Over 100 subtypes of HPV may exist in humans, with each belonging to either a low-, intermediate-, or high-risk subtype. An important distinguishing characteristic between these subtypes is the binding affinity of the E6 and E7 gene products for the tumor suppressors p53 and Rb, respectively (6-8). These early gene products of the low-risk HPVs, such as HPVs 1, 6 and 11, demonstrate no such binding, and are associated with the common wart, genital warts, and condylomata acuminata. In contrast, the transforming, high-risk HPVs, including HPV 16, 18, 31, 33, and 45 are associated with carcinoma development.

It is estimated that approximately 95% of cervical carcinomas may be associated with high-risk HPVs, most notably HPV 16 and 18 (9-12). Although both high- and low-risk subtypes may be associated with grade I cervical intraepithelial neoplasia (CIN), most CIN III (and many CIN II) lesions are associated with high-risk HPV types. Even though an accurate estimate of the incidence rate of HPV is not available, recent reports indicate the incidence of HPV has increased over the past few decades (10). Prevalence rates vary widely, depending upon the detection method utilized (with higher prevalence rates reported in those studies using PCR-based technologies) and the population examined, with current estimates ranging between 5% and 50% in women with a normal pap smear. Most infected women become HPV negative (i.e. HPV DNA undetectable) within two years; however, that subset of women who become persistently infected have an increased likelihood of developing CIN III lesions and cervical carcinoma (13-16).

Although HPV's causative role in cervical cancer is now well established, the association between HPV infection and a growing number of carcinomas in other tissues is less clear as the PCR-based technology used to detect a large variety of HPV types is extremely sensitive and prone to false positives, possibly attributable to incidental HPV contaminants. Despite these difficulties, faced years ago by those attempting to demonstrate the association between cervical cancer and HPV, a growing body of evidence supports the contention that HPV's role in epithelial cell transformation may not be limited to cervical cancer. For example, the association between HPV infection and a subset of head and neck squamous cell carcinomas (HNSCC) is now well established (17-20). Therefore, therapeutic vaccines being developed for use in cervical cancer patients may be useful in the treatment of some HNSCC patients.

Non-melanoma skin cancers (NMSC) are the most prevalent malignancies in the caucasion population worldwide. HPV has been implicated in the cutaneous malignancies associated with the rare and inherited disorder, epidermodysplasia verruciformis (EV). Recent studies have demonstrated that a significant number of NMSC in both immunocompetent and immunosuppressed individuals may be associated with various HPV types (21-23). Several groups have demonstrated that HPV gene products expressed under control of a keratinocyte-specific promoter in transgenic mice lead to the development of skin cancers in older mice (24), or in transgenic mice exposed to chemical carcinogens (25) or UV-irradiation (Wilcox et. al., unpublished data). The use of such transgenic models may not only help to elucidate the molecular events required for the malignant transformation of the HPV infected cell, but they may also improve our understanding of the immune response generated at each stage in this multi-step process (26).

The association of HPV infection and transitional cell carcinoma (TCC) of the bladder has been reported (27-31), although not all attempts to demonstrate the presence of HPV in neoplastic tissue have been successful (32-34). Cases of rapidly progressive multifocal TCC of the bladder have been reported in immunosuppressed transplant recipients, suggesting HPV's role in TCC of the bladder in immunocompromised patients (35, 36). One report has implicated HPV16 in a number of squamous cell carcinomas of the lung on the island of Okinawa (37, 38). A few studies would seem to support this finding (39-41), although others have failed to implicate HPV in lung cancer (42). Perhaps even more controversial is the recently proposed association between HPV and squamous cell carcinoma of the esophagus (43).

Data implicating a relatively few HPV subtypes in the neoplastic transformation of epithelial cells at a wide range of anatomic sites, not limited to the uterine cervix, implies that the vaccine strategies currently employed in cervical cancer patients may be beneficial in a number of other malignancies. The goal of this review is to summarize the current status of therapeutic HPV vaccine strategies, emphasizing the potential challenges posed by T cell tolerance and immunologic ignorance, and the role of T cell costimulation in the setting of tumor immunotherapy.

3. IMMUNOTHERAPEUTIC STRATEGIES: DENDRITIC CELL AND TUMOR CELL BASED VACCINES

Preclinical and clinical studies have shown the utility of prophylactic and therapeutic vaccines in the setting of HPV-associated disease (44). Whereas the goal of any prophylactic vaccine, which is beyond the scope of this review, is to prevent HPV infection by the induction of an appropriate humoral and cell-mediated immune response capable of producing neutralizing antibodies, the goal of a therapeutic vaccine is the eradication of premalignant and malignant HPV-associated lesions.

Several lines of evidence support the notion that the induction of a potent cell-mediated immune response may be capable of controlling HPV-associated malignancies. First, immunosuppressed patients have an increased incidence of both premalignant and malignant lesions of the cervix (45-50). Furthermore, spontaneous regression of HPV-associated lesions has been observed and is associated with a cell-mediated immune response (14). Tumor infiltrating lymphocytes may be demonstrated in cervical lesions by immunhistochemistry and HPV-specific T cells have been demonstrated, not only in peripheral blood, but in tumor draining lymph nodes and the tumors of CIN and cervical cancer patients (51). Not surprisingly, the downregulation of HLA class I molecules during the progression of dysplastic lesions to neoplastic lesions suggests that immune evasion may be an important contributing factor in disease progression (52, 53). Collectively, this data provides a strong rationale for the addition of T-cell based immunotherapies to our arsenal in the fight against HPV-associated malignancies.

As both the E6 and E7 gene products are required for the maintenance of the transformed phenotype, these viral proteins are attractive targets for immunotherapy. To date, many class I restricted peptides have been identified and have been shown to be immunogenic in both mouse models and phase I/II human trials (54-64). Studies performed in mice have demonstrated that peptide immunization may confer protection against a subsequent challenge with an HPV-transformed tumor. However, the same approach, although capable of generating a detectable CTL response, was incapable of treating established tumors. Van Elsas *et. al.* recently demonstrated that immunotherapy of a murine melanoma was dependent upon a CTL response, whereas tumor rejection was observed following a prophylactic vaccine in the absence of $CD8^+$ T cells (65). These studies warrant a degree of caution when attempting to extrapolate data obtained from prophylactic vaccine studies to a therapeutic setting. The present challenge then, is to develop immunotherapeutic strategies capable of treating established tumors.

It may be argued that dendritic cells (DCs) are not only the most potent professional APC, undoubtedly due to their unique ability to process and present antigen, express costimulatory and adhesion molecules, and secrete proinflammatory cytokines; but they also play a pivotal role in the initiation, regulation, and maintenance of a cell-mediated anti-tumor immune response (66). Although DCs represent less than 1% of peripheral blood leukocytes, sufficient numbers of DCs may be obtained for use in immunotherapy protocols following the *ex vivo* expansion of DCs in IL-4 and GM-CSF from monocytes or monocyte ($CD34^+$) precursors. Phenotypically immature DCs, although less efficient APCs, efficiently phagocytose apoptotic and necrotic cells and macropinocytose soluble proteins. Upon maturation, DCs become more potent APCs, capable of presenting antigenic peptides to $CD4^+$ T cells and exogenous antigenic peptides to $CD8^+$ T cells via "cross-priming." The upregulation of various costimulatory molecules and an increased ability to secrete inflammatory cytokines are hallmarks of a phenotypically mature DC. Further understanding of basic DC biology may prove useful in the determination of the "maturation state" which is optimal for any given DC-based immunotherapy. Furthermore, the ancestry of DCs used in immunotherapy, whether lymphoid or myeloid, or DC1 or DC2, may be important considerations (67-69). For example, Mohamadzadeh et. al recently demonstrated that IL-15 and GM-CSF could generate DCs similar to Langerhans cells from peripheral blood. This is an exciting finding, as these cells were more potent stimulators of a CTL response when compared to DCs generated in IL-4 and GM-CSF (70).

Many DC-based immunotherapies have been attempted in mouse models, some of which have been applied in various clinical trials with a wide range of different malignancies. One approach is to load DCs *in vitro* with tumor antigens, whether in the form of antigenic peptides, recombinant protein, RNA or DNA encoding a known tumor antigen, tumor-derived RNA, tumor cell lysates, tumor-derived apoptotic bodies or exosomes, or CVLPs (71-84). Encouraging results have been obtained using HPV16 E7 peptide-pulsed DCs in mouse models. For example, peptide-pulsed DCs not only protect mice against subsequent challenge with an HVP16 E7-expressing tumor, but this approach also eradicated large established tumors (84-86).

Strategies, which do not require any prior knowledge of a patients MHC haplotype, or even identification of a relevant tumor antigen, would seem most attractive. One approach includes the use of autologous or allogeneic, irradiated, whole tumor cell vaccines transduced to express cytokines, such as GM-CSF or IL-12, or costimulatory molecules, like B7-1. The ability of tumor cell vaccines to induce a CTL response and confer protection against a tumor challenge has been well documented in the literature. These approaches have only recently been applied to HPV in preclinical studies (87, 88). Although this approach may have its advantages, especially in those malignancies in which tumor antigens have not yet been identified, this approach seems relatively less attractive in the setting of cervical cancer in which tumor antigens are well characterized. Furthermore, although tumor cell vaccines may be capable of conferring protection against a subsequent tumor challenge, these vaccines are considerably less effective in the therapeutic setting. With that in mind, the fusion of either autologous or allogeneic tumor cells with autologous DCs (89, 90), or loading of DCs with tumor cell derived products (e.g. heat shock proteins, exosomes, apoptotic bodies, RNA, or cell lysates) may not be unreasonable. Alternative approaches may include the delivery of DCs to tumors *in vivo*, or the mobilization of DCs using Flt-3 ligand or GM-CSF and IL-4 (91-96).

4. T-CELL TOLERANCE, IMMUNOLOGIC IGNORANCE AND INCOMPETENCE

The imposition of self-tolerance, both centrally and peripherally, may pose a significant barrier to the stimulation of a potent antitumor immune response capable

of eradicating an established tumor or preventing tumor recurrence following conventional therapeutic modalities. (Although HPV clearly represents a "foreign" antigen, HPV infection is not usually associated with an inflammatory response. Furthermore, HPV is a non-lytic virus, and HPV-infected cells, once desquamated from the epithelial surface may be inaccesible to antigen presenting cells. This lack of "danger," and likely sequestration of antigen in desquamated epithelial cells may justify the comparison between a self-antigen expressed in the periphery and HPV antigens.) Therefore, the potential mechanisms responsible for the maintenance of self-tolerance and the methods that might be useful in breaking tolerance may be worth careful consideration in the cancer immunotherapy setting.

4.1. Tolerance

There are undoubtedly many factors which control the balance between immunity and tolerance in the periphery, many of which are poorly understood. Such mechanisms fall within a broad spectrum, ranging from T cell deletion (97-102), anergy or activation-induced nonresponsiveness (AINR) (103-107), to outright immunological ignorance or indifference (108-110), all of which may be operational in a given patient expressing multiple tumor associated antigens (111). T cell deletion has been observed in both autoimmune and tumor models (97-102) and is thought to be the result of a high antigen load, or in the case of a CTL response, a lack of $CD4^+$ T cell help (100, 112, 113).

In contrast to deletional mechanisms, T cell anergy involves the induction of a functionally unresponsive state in antigen-specific T cells. T cell anergy has been classically defined in $CD4^+$ T cells and may result from the presentation of antigen in the absence of the appropriate costimulatioin, leading to a defect in IL-2 secretion upon antigenic restimulation (114-116). Functional unresponsiveness in $CD8^+$ T cells, recently described as activation-induced nonresponsiveness, is similar to the clonal anergy observed in $CD4^+$ T cells, in that it involves an inability of $CD8^+$ T cells to secrete IL-2 and proliferate upon reencounter with antigen (117). However, in contrast to clonal anergy, AINR is thought to occur following TCR engagement and costimulation (117-120). In addition, AINR in $CD8^+$ T cells is independent of CTLA-4 stimulation (117, 120). AINR is reminiscent of the "split anergy" observed in CTL clones following activation in the absence of costimulation . Although the cells were unable to secrete IL-2 and proliferate upon restimulation, the ability to lyse target cells (121) and secrete IFN-γ (wilcox *et. al.* unpublished data) remained intact. This dichotomy may be explained by the recent observation that AINR involves the defective upregulation of ERK and p38, MAP kinases essential for IL-2 production, whereas lytic function may be maintained due to the retention of the TCR-induced calcium flux (118). Anergy or AINR of $CD4^+$ and $CD8^+$ T cells, respectively, has been observed in mouse tumor models. Given the subtle differences in clonal anergy and AINR, the conditions needed to break the one may be innefective in reversing the other.

Systemic immune suppression may also play an important role in preventing an antitumor immune response. Many tumors, including cervical cancer, may result in systemic immunosuppression. Suppressor T cells may also play a role in the maintenance of self-tolerance. Sakaguchi *et. al.* made the important observation that depletion of $CD4^+CD25^+$ T cells from $CD4^+$ cells prior to adoptive transfer into nu/nu recipients led to autoimmune disease (122, 123). Subsequent work has clearly identified this subpopulation of $CD4^+$ T cells as important suppressors of self-reactive T cells *in vivo* and *in vitro* (124). The role of $CD4^+CD25^+$ suppressor cells in tumor immunity has been demonstrated by the enhanced anti-tumor immune response observed upon their depletion *in vivo* or upon their depletion *in vitro* prior to adoptive transfer of autologous T cells obtained from tumor-bearing mice (125).

4.2. Immunologic ignorance

Immunologic ignorance may be particularly relevant when considering HPV. As previously mentioned, once epithelial cells are infected by HPV, viral antigens are likely sequestered from roaming APC's and the infection itself fails to elicit an inflammatory response. The coexistence of naïve, fully functional antigen-specific T cells and a peripheral antigen has been described as immunological ignorance. However, ignorance should be distinguished from the situation in which a T cell response is generated, either naturally or following immunotherapy, but the response is incapable of suppressing tumor growth, even though the tumor cells are susceptible targets. We will designate the latter situation in which T cells have been primed, but fail to control tumor outgroth as immunologic incompetence.

Ignorance was first demonstrated by Ohashi *et. al.* using double transgenic mice expressing both the LCMV-GP under control of the rat insulin promoter (RIP) and an LCMV-GP TCR transgene. The LCMV-GP specific T cells in these mice, while not deleted or anergized, ignored the viral antigen expressed on the islet β cells (108). This immunologic ignorance could be broken either by infection with the virus or by the local expression of B7-1 on islet cells (126). A similar study performed in mice expressing both B7-1 and TNFα in islet cells demonstrated the importance of both local inflammation and costimulation on the induction of autoimmunity (127). Similar experiments were performed in which a fragment of the SV40 large T antigen was expressed in the islets of mice which also expressed a TCR specific for an antigenic peptide of the large T antigen. In these mice, the antigen was ignored; however, the adoptive transfer of fully activated specific T cells into mice expressing the antigen did not result in autoimmunity (128). Similarly, ignorance has also been observed in transgenic mice expressing the E6 and E7 oncogenes of HPV16 under the control of a keratin-specific promoter (129). In these mice, the E6 and E7 gene products were expressed in all keratinized epithelial cells. Cultured keratinocytes from these mice could be lysed by E7 specific CTLs *in vitro*. Although immunization with an E7-derived peptide conferred protection against a subsequent challenge with a tumor

expressing the E7 gene product, no skin pathology was noted in these transgenic mice. These studies demonstrate that although systemic ignorance may be broken upon immunization with the appropriate antigen , this does not guarantee that the CTL response generated will be sufficiently potent to mediate autoimmunity.

Immunologic ignorance has also been described in tumor models. For instance, Wick *et. al.* showed that Ag104 tumor cells transfected with L^d could grow in 2C mice, although L^d-expressing skin was rejected (110). Similarly, transgenic mice expressing a TCR specific for the nonmutated tumor antigen P1A cannot reject the P1A positive J558 plasmacytoma (130). While the evidence for immunological ignorance is clear, the mechanisms responsible for maintenance of the ignorant state are poorly understood.

One obvious factor which is likely important in the maintenance of immunological ignorance is the dose of antigen (131). This has been demonstrated in transgenic mice expressing either high (RIP-Ova_{hi}) or low (RIP-Ova_{lo}) levels of ovalbumin under the control of the rat insulin promoter (112). In these mice, the RIP-Ova_{hi} deleted antigen-specific OT-1 cell *in vivo*. In contrast, Ova was completely ignored in the RIP-Ova_{lo} mice. Importantly, both transgenic lines were susceptible to islet β cell destruction following the adoptive transfer of activated OT-1 cells (132). It was also noted that in chimeric RIP-Ova_{hi} mice incapable of cross-presenting antigen, OT-1 cells were no longer deleted, but became ignorant. The latter observation highlights the potential influence of cross-priming in controlling the balance between tolerance and immunity.

Direct priming and cross-priming may both play redundant roles in tumor immunity (133, 134). However, not all tumors may be associated with cross-presentation of tumor antigens. For example, it has been shown that in various tumors expressing LCMV-GP, the LCMV-GP is not cross-presented to antigen specific T cells *in vivo* (135, 136). Given that all the tumors used in these studies were transfected with the same antigen, antigenic epitopes derived from this antigen may not be properly processed and presented by the immune proteasome. Therefore, to conclude from these studies that cross-priming is extremely rare may be an unjustified extrapolation. Despite this shortcoming, it was clearly demonstrated that the ability of a tumor to directly initiate a T cell response was associated with that tumor's ability to metastasize to secondary lymphoid organs, like the tumor draining lymph node. It was also noteworthy that tumors, which metastasize to the draining LN, may be encapsulated and thus remain inaccessible to naïve T cells. Therefore, a lack of cross-priming, whether or not that is determined by the dose of antigen or the anatomic site of the tumor (137), and/or a lack of direct-priming, presumably due to a tumors inability to gain access to naïve T cells in secondary lymphoid organs, or a tumor's ability to subvert these modes of antigen presentation, may explain immunologic ignorance.

Recent studies have also demonstrated the importance of secondary lymphoid organs in T cell activation following the cross-presentation of antigen. Bai *et. al.* demonstrated that following T cell activation, either by direct or indirect antigen presentation, P1A specific T cells underwent a phenotypic change, signified by the upregulation of HSA, at the tumor site. This change was also observed in MHC class I deficient tumors, suggesting the presence of cells, like macrophages or dendritic cells, capable of cross-presenting antigen to activated T cells. In contrast, T cell activation was not observed when naïve T cells were injected directly into the tumor site. Therefore, secondary lymphoid organs, but not the tumor microenvironment, may be permissive for the productive cross-presentation of tumor-associated antigens to naïve T cells. This limitation may not be restricted to tumors, as even allogeneic, vascularized organ transplants may be ignored in mice lacking secondary lymphoid organs (138). Although the mechanism responsible for this difference is unknown, several hypotheses may be offered.

First, the tumor microenvironment may not be sufficiently "dangerous" to sufficiently mature APCs involved in the cross-presentation of antigen (139, 140). Alternatively, the tumor may actively suppress APC maturation and T cell activation via the secretion of soluble factors like TGF-β or VEGF (141-144). A recent study has shown that in a collagen matrix, T cells interacted only transiently with antigen-presenting DCs (145). Therefore, collagen present in the periphery, including mucosal surfaces, may interfere with formation of the immunological synapse. In contrast, the absence or shielding of collagen by stromal cells in secondary lymphoid organs may favor an immunologically productive encounter between a naïve T cell and an antigen-presenting cell (146). Chemokine gradients present *in vivo* may also promote T cell ignorance in the tumor setting. Bromley et al. showed that "dominant chemokine gradients," including SLC may prevent immunological synapse formation (147). Therefore, in the absence of inflammation at the tumor site, a naïve T cell may exit the periphery, despite the presence of antigen, in response to an SLC gradient. In contrast, inflammatory chemokines like MIP-3β produced during an inflammatory response may favorably compete with any "dominant chemokine gradient," promoting immunological synapse formation and T cell activation in the periphery.

4.3. Immunologic incompetence

In contrast to immunological ignorance, incompetence is characterized by the activation of antigen-specific T cells; however, this T cell response is incapable of controlling tumor outgrowth. For example, Ag104 tumor cells transfected with L^d grew in 2C mice even though L^d-expressing skin grafts were rejected. In this model, incompetence could only be overcome if mice were preimmunized with L^d-expressing skin grafts and tumor cells expressed both B7-1 and CD48 (110). Furthermore, the P1A positive plasmacytoma J558 grow in transgenic mice expressing a TCR transgene specific for the P1A antigen. However, tumor cells expressing either B7-1 or B7H were rejected (130, 148, 149). Interestingly, in mice inoculated with both J558-neo and J558-B7-1, only the B7-1 expressing tumor cells were eliminated, further implicating a lack of local costimulation in the maintenance

of immunological incompetence. Like ignorance, the mechanisms responsible for immunological incompetence are not yet fully understood.

The ability of activated T cells to gain access to the tumor is an important consideration. Transgenic mice expressing the SV40 large T antigen in islet β cells of the pancreas eventually leads to insulitis and the development of solid tumors (128). Interestingly, even when these mice were crossed with mice expressing a Tag-specific TCR transgene, the solid tumors were not infiltrated with T cells in the double transgenic mice, despite the presence of insulitis in adjacent tissue. Even the expression of B7-1 by islet β cells, although sufficient for the development of diabetes, did not prevent the outgrowth of solid tumors. Although these tumors maintained expression of the large T antigen and were not infiltrated by T cells, the tumors had lost expression of B7-1 (150). However, in this model, T cell extravasation into the tumor site was observed when *ex vivo* activated T cells were adoptively tranferred into mice which had previously received a high dose of whole body irradiation. Ionizing radiation has been shown to upregulate endothelial adhesion molecules which may be extremely important in the recruitment of effector T cells to the tumor site (151-153). These findings may be relevent given the observation that many tumors may regulate the expression of adhesion melecules by endothelial cells present within the tumor, thus preventing T cell extravasation at the tumor site (154, 155).

T cells capable of extravasating at the tumor site may not remain there long enough to completely eradicate a tumor. This has been observed in a mouse model in which the adoptive transfer of antigen-specific T cells was incapable of controlling the growth of lymphoma cells in the peritoneum. This was the result of T cell migration away from the tumor site into secondary lymphoid organs. After migration away from the tumor site, the T cells appeared to have undergone AINR, as they were capable of killing tumor targets *in vitro*, even though they were unable to proliferate upon restimulation (119).

The T cell repertoire, including the avidity of antigen specific T cells for their cognate antigen, may also be an important factor influencing immunological incompetence. This may be especially true in situations in which a tumor-associated antigen is presented in the thymus and central tolerance enforced (156). Alternatively, only T cells with a low avidity for a tumor-associated antigen may escape negative selection (157). Many studies have implicated costimulation at the tumor site as an important factor in immunological incompetence (110, 148, 149). Although costimulation itself may be an important factor in breaking immunological incompetence, it is also possible that the expression of costimulatory molecules on tumor transfectants may serve to increase the avidity of the T cell/tumor cell interaction.

4.4. HPV transgenic models

Whether or not HPV-associated malignancies induce tolerance is a difficult question given the problems associated with accurately measuring an antigen-specific T cell response in cancer patients. However, work performed in transgenic mice expressing HPV antigens in peripheral tissues, including skin , suggest that multiple modes of self-tolerance may be enforced. In these models, HPV16 E6 and/or E7 oncogenes are expressed under the control of promoters, such as the keratin 14 promoter, which restrict transgene expression to epithelial surfaces. As many of these mice also express the transgene in the thymus, both central and peripheral tolerance may be important (158). However, studies performed in radiation bone-marow chimeras have demonstrated that transgene expression in the periphery alone is sufficient to tolerize E7-specific CTLs (159). Studies performed in various lines of transgenic mice provide evidence for anergy (158, 160), ignorance (129, 161-163), and incompetence (129, 164). Interestingly, CTL tolerance could be maintained in transgenic mice in which antigen specific T helper cells were not tolerized or showed evidence of having been previously activated (165, 166). Therefore, the status of any B cell or $CD4^+$ T cell response may not be a reliable indication of the immune status of tumor-specific CTLs.

One line of transgenic mice frequently develop SCC of the skin and lenticular tumors upon aging. In younger mice, E7 specific T cells appear to ignore the antigen, however, development of DTH responses to E7 was observed in older mice with skin pathology. Therefore, immunological ignorance may be prominent early in disease progression (i.e. low grade CIN), but an observable immune response may be generated later as the disease progresses, potentially inducing an inflammatory response or metastasizing to secondary lymphoid organs (161). It should be noted that the depletion of $CD4^+$ T cells in these mice was associated with a reduced incidence of skin pathology, thus raising the possibility that any immune response may precede noticeable skin pathology (163). Alternatively, loss of $CD4^+CD25^+$ suppresor T cells in these mice may result in the generation of a more robust cell-mediated immune response capable of limiting skin disease. Further studies in these mice should continue to shed light on the mechanisms of tolerance in HPV-associated malignancies and the potential methods capable of preventing tolerance induction or reversing tolerance once established.

4.5. Tolerance, immunologic ignorance and incompetence in cervical cancer

As in some of the transgenic mouse models, both T helper cell and CTL responses are detectable in CIN and cervical cancer patients (51, 167-173). However, the relevance of these findings in determining the significance of tolerance in patients is confounded by several variables. First, these studies inevitably involve the *in vitro* culture of patient PBMC in the presence of IL-2, a procedure which may reverse T cell anergy. As already noted, the detection of a $CD4^+$ T cell proliferative response may not be associated with a CTL response. Furthermore, T cell responses in patients with advanced disease may be recent occurences associated with a high tumor burden and antigen load, or even an ongoing infection (170). Therefore, the detection of a T cell response in a patient with advanced disease may reveal very little about the

putative immune response earlier in the disease course. Finally, most of these studies use PBMCs as a source of T cells; however, the frequency of tumor-specific T cells may be significantly higher in the tumor-draining lymph node or the tumor site (174). Therefore, the absence of a detectable T-cell response in peripheral blood may be uninformative, and most certainly cannot be interpreted as evidence for immunological ignorance.

Despite these pitfalls, evidence exists to suggest that self-tolerance may be as important a consideration in human disease as it is in both mouse tumor models and in studies performed in HPV transgenic mice. As has been observed in a variety of tumor types, HPV-associated malignancies may be associated with systemic or local immunosuppression. A variety of studies have shown that HPV-derived proteins may be immunosuppressive. This may be attributed to their ability to abrogate signaling mediated by type I interferons (175), to inhibit IL-18 induced IFN-γ production (176), or to inhibit the secretion of IFN-α by APCs (177). Furthermore, HPV16 E6 oncoprotein may stimulate the release of VEGF at the tumor site, thus promoting angiogenesis and inhibiting DC maturation (178, 179). In fact, VEGF expression is a negative prognostic indicator following radiation therapy for cervical cancer (180). The presence of DCs within the tumor site is a positive prognostic indicator in a variety of different malignancies (181), including HPV-associated malignancies (182-185). The lack of DC infiltration within preneoplastic and neoplastic lesions may prevent the cross-presentation of tumor-associated antigens, thus preventing the induction of a cell-mediated immune response, favoring immunological ignorance. In contrast, antigen presentation by tumor-associated APCs may result in the induction of tolerance (186-190). This may be attributed to the secretion of a variety of immunosuppressive cytokines by tumor cells, including IL-10, TGF-β, or VEGF. Interestingly, an association between smoking and cervical cancer has been recognized for many years (191). It is unclear whether that may be attributed to the association between smoking and immune suppression, including a reduction in the density of Langerhans cells within the cervix (192). Furthermore, a persistent HPV infection may be associated with local infiltration of B cells capable of presenting HPV-associated antigens to CTL in a tolerogenic fashion (193-195) or may result in the generation of a Th2/Tc2 deviated immune response (196-198).

Collectively, the data enumerated above provides strong circumstantial evidence that various modes of immune tolerance, including immunological ignorance, may predominate at various stages of disease progression in HPV-associated malignancies. Therefore, the design of strategies capable of preventing and reversing tolerance and immunologic ignorance may be important additions to the tumor immunologist's arsenal.

4.6. Tolerance and immunotherapy

Tolerance is also an important consideration in the immunotherapy setting. For example, many mouse and human studies have demonstrated the great potential of peptide-based immunotherapeutic strategies; however, caution may be warranted as studies have shown that some peptides administered in IFA, due to their pharmacokinetics, may actually induce tolerance, thus promoting tumor outgrowth (199, 200). Consequently, studies in HLA congenic mice may be useful, not only for identifying immunogenic peptides, but in screening these peptides for their ability to induce tolerance. An alternative approach may be the development of strategies which prevent tolerance, thus converting an otherwise tolerogenic peptide into an immunogenic vaccine. For example, tolerization may be avoided by the presentation of antigenic peptides by peptide-pulsed DCs.

Chimeric VLPs, including HPV 16 E7/L1 or L2 fusions elicit an E7-specific CTL response capable of protecting immunized mice against subsequent challenge with E7-expressing tumors (201, 202). In addition, CVLPs may prevent the induction of tolerance. Nieland *et. al.* recently demonstrated that immunization of mice with a peptide derived from the nonmutated tumor antigen P1A in IFA induced tolerance leading to the outgrowth of a regressor P815 cell line. However, immunization of mice with a CVLP containing the same P1A peptide induced a protective immune response (203). It is unclear whether or not this may be explained by the ability of CVLPs to upregulate costimulatory molecule expression and cytokine secretion in immature dendritic cells which have taken up CVLPs (71, 204).

Although DCs have been exploited in the immunotherapy setting with promising results, the observation that DCs, particularly lymphoid DCs, may play a role in the induction of self-tolerance raises the possibility that vaccine preparations including DCs may be further optimized by the removal of any tolerogenic cells (205-211). For that reason, Ruedl *et. al.* compared the ability of myeloid and lymphoid DCs to prime a CTL response *in vivo* (212). Although both DC subsets were capable of eliciting a protective anti-viral CTL response, the possibility that a DC based vaccine may induce tolerance cannot be excluded given the different DC lineages and maturation states. The method used to load DCs with tumor antigens may be an additional consideration. For instance, necrotic tumor cells have been shown to be more immunogenic than apoptotic cells (213, 214), an observation that may be attributed, at least in part, to the ability of necrotic cells to stimulate DC maturation (215-218). In addition, DCs loaded with apoptotic tumor cells may induce tolerance (206, 207). The notion that DC based approaches to tumor immunotherapy are immune to the challenges posed by T cell tolerance may be naïve.

5. T-CELL COSTIMULATION IN IMMUNOTHERAPY

T cells require both antigen-dependent and independent (i.e. costimulatory) signals to become fully activated. Therefore, the administration of a tumor-associated antigen may be insufficient to eradicate established tumors in the absence of appropriate costimulation. On the other hand, immunogenic tumors capable of priming antigen-specific T cells may regress

after those T cells receive an appropriate costimulatory signal. This may be achieved in a number of ways, including the expression of an appropriate costimulatory ligand by a tumor or by the systemic administration of agonistic monoclonal antibodies against any one of a number of costimulatory molecules. Manipulation of the growing repertoire of T cell costimulatory molecules may enable tumor immunologists to modulate the antitumor immune response in such a way so as to overcome some of the barriers, including those mentioned above, which prevent the induction and maintenance of a potent antitumor cell-mediated immune response.

Although the manipulation of many costimulatory receptors, including CD40 (219-223), OX-40 (224, 225), and CTLA-4 (222, 226-230), have already been demonstrated to play a role in the enhancement of the antitumor T cell response and/or in the prevention and reversal of tolerance, the remainder of this review will emphasize the role of 4-1BB (CD137) costimulation in the tumor immunotherapy setting.

5.1. 4-1BB (CD137) immune regulation

4-1BB is a member of the tumor necrosis factor receptor superfamily (TNFRSF) that is expressed in an activation-dependent fashion on $CD8^+$ and $CD4^+$ T cells, intraepithelial lymphocytes (IELs), NK cells, eosinophils, and endothelial cells (231-238). *In vitro* studies have demonstrated that both agonistic anti-4-1BB mAb and 4-1BB ligand (4-1BBL) can costimulate proliferation and cytokine secretion in both $CD4^+$ and $CD8^+$ T cells (239-241). However, studies performed *in vivo* suggest that 4-1BB may play a more prominent role in the generation of a CTL response than in a T-helper cell response. In fact, administration of anti-4-1BB mAb in mice may induce anergy in $CD4^+$ T cells (242) or may even promote activation-induced cell death (243, 244). In contrast, the importance of 4-1BB costimulation in a purely $CD4^+$ T cell response has been demonstrated in a graft versus host disease model (245). Therefore, the importance of 4-1BB costimulation in a $CD4^+$ T cell response is not yet entirely clear; however, the importance of 4-1BB/4-1BBL interactions in the generation of a fully competent CTL response has been confirmed in studies performed in 4-1BBL deficient mice (246-248). From the tumor immunologist's point of view, the ability of agonistic anti-4-1BB mAb or 4-1BBL to eradicate established tumors in mouse models is of great interest (137, 249-255).

Although anti-4-1BB mAb may eradicate immunogenic tumors, the same treatment is considerably less effective or completely ineffective in the treatment of poorly immunogenic tumors. We have recently described two tumors expressing the HPV-16 E6 and/or E7 oncogenes. Both tumors may be lysed *in vitro* by E7(49-57)-specific T cells. However, *in vivo*, only one of the tumors is immunogenic, as demonstrated by both tetramer analysis and *in vitro* ^{51}Cr-release assays for CTL activity. Not surprisingly, administration of anti-4-1BB mAb in tumor-bearing mice was capable of eradicating this tumor. In contrast, E7-specific T cells were neither deleted nor tolerized in mice bearing the second, less immunogenic tumor. In fact, no evidence of a T cell response to this tumor was observed in tumor-bearing mice, suggesting that the tumor may be ignored. Administration of anti-4-1BB mAb in these mice was completely ineffective. As T-cell priming is required to upregulate 4-1BB expression on antigen-specific T cells, the inability of anti-4-1BB mAb to overcome immunologic ignorance to this tumor may not be surprising. Although vaccination of tumor-bearing mice with the E7(49-57) peptide in incomplete Freund's adjuvant was incapable of eradicating established tumors, even though a CTL response was detected following vaccination, administration of anti-4-1BB mAb following vaccination was capable of overcoming immunologic ignorance to an extent capable of eradicating established tumors (256). This novel approach, which we have dubbed COPP (Costimulatory anti-4-1BB mAb Plus Peptide), was also successfully applied in lung metastases models using both B16-F10 and TC-1 tumor cells. It should also be noted that anti-4-1BB mAb was also affective in treating tumors when combined with an irradiated tumor-cell vaccine. Therefore, use of anti-4-1BB mAb may not be limited to peptide-based vaccines, but may be a useful addition to antigen-based immunotherapies, including tumor-cell vaccines and DC-based approaches. Immunotherapy with antigenic peptides and anti-4-1BB mAb may be an effective form of therapy in HPV-associated malignancies, many of which may be immunologically ignored.

Following COPP treatment, a significant increase in the number of E7-specific tumor-infiltrating lymphocytes (TILs) was observed at the site of regressing tumors. Although the mechanism involved is not yet known, many attractive hypotheses may be offered. First, 4-1BB stimulation may enhance the ability of T cells to migrate to the tumor site. Although the effect of 4-1BB stimulation on 4-1BB-expressing endothelial cells is unknown, the upregulation of adhesion molecules important in T cell trafficking is a possibility. Alternatively, 4-1BB costimulation has been shown to enhance the ability of T cells to adhere to components of the extracellular matrix in a β1-integrin-dependent fashion (257). Furthermore, 4-1BB stimulation may upregulate 4-1BB expression via a positive feedback loop (258, 259), and murine 4-1BB is capable of binding fibronectin (260). In both cases, any enhancement in a T cell's ability to adhere to the extracellular matrix may improve the cell's ability to traffic to a tumor or remain at the tumor site once there. Following peptide immunization, a significant increase in the clonal burst size was noted in the draining lymph nodes of mice following anti-4-1BB mAb treatment. Therefore, any increase in the number of TILs may be a mere reflection of this increase in clonal burst size. Finally, the ability of 4-1BB costimulation to alter the expression patterns of chemokines and their receptors on antigen-specific T cells has not been investigated, but may be a possibility worthy of consideration, as other costimulatory members of the TNFRSF have been shown to alter the expression of various chemokines and their receptors (261, 262).

Second, 4-1BB stimulation has been shown to prevent AICD (263-265). Therefore, the increased number

of TILs observed after treatment may be explained by the prolonged survival of these T cells. Alternatively, 4-1BB stimulated T cells may escape AINR. AINR may be observed in the later phase of a CTL response that receives insufficient help from $CD4^+$ T cells. T cells which escape AINR may be capable of proliferating upon encountering cognate antigen at the tumor site. In fact, we have demonstrated that the intravenous administration of high doses of antigenic peptide may induce AINR in antigen-specific $CD8^+$ T cells. However, administation of anti-4-1BB mAb prevented the induction of AINR in this model. Furthermore, administration of peptide and anti-4-1BB mAb ten days following the induction of AINR was capable of *reversing* nonresponsiveness (Wilcox et. al., unpublished data). To our knowledge, anti-4-1BB mAb administration is the only therapy capable of reversing established AINR *in vivo*. The ability to reverse AINR may be extremely valuable in the immunotherapy setting. In summary, 4-1BB costimulation may promote the expansion, survival, and/or migration of antigen-specific T cells to sites of tumor growth, and may therefore represent an exciting new approach in the immunotherapy of HPV-associated malignancies.

Depletion studies in COPP-treated mice revealed that treatment was independent of $CD4^+$ T cells, but was NK-cell dependent. Preliminary studies have revealed that 4-1BB-stimulated NK cells may play an important immunoregulatory role, raising the possibility that 4-1BB-stimulated NK cells may be able to compensate, at least in part, for a deficient $CD4^+$ T cell response.

As previously described, many mechanisms may be invoked to explain immunological ignorance in the setting of HPV-associated malignancies. One potential mechanism, is the inefficient processing and presentation of HPV antigens by bone-marrow derived APCs, like the dendritic cell. Therefore, our recent observation that murine DCs express 4-1BB raised the possibility that 4-1BB stimulation may somehow alter the phenotype or function of the dendritic cell. With that in mind, we isolated splenic DCs from mice which had received anti-4-1BB mAb and were able to demonstate that these DCs were more potent stimulators of proliferation in both allogeneic and antigen-specific T cells (266). The mechanism involved in this apparent upregulation of the DCs ability to stimulate T cells following 4-1BB stimulation is currently under investigation and raises the possibility that the successful eradication of established tumors following anti-4-1BB mAb treatment may require 4-1BB stimulation of DCs. Although much remains to be learned, the ability of anti-4-1BB mAb treatment to, not only modulate the innate and adaptive immune responses, but also prevent and reverse AINR, are findings which, we believe, may prove useful in the treatment of HPV-associated malignancies.

6. CONCLUSION

With the identification of a vast number of tumor antigens has come great progress in the rational design of antigen delivery strategies capable of eliciting a detectable T cell response against HPV-associated antigens. Unfortunately, the goal of tumor immunotherapy is not the induction and subsequent detection of a tumor-specific T cell response, but tumor eradication and tumor-free survival. While the delivery of tumor antigens may induce a cell-mediated immune response, the regulation and maintenance of that response may be critical in tumor eradication. It remains to be determined whether or not more and better antigens alone are capable of achieving that goal. If not, immune regulation may be the next frontier in tumor immunotherapy.

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8. REFERENCES

1. Lorber, B.: Are all diseases infectious? *Ann Intern Med* 125, 844-51. (1996)
2. zur Hausen, H.: Viruses in human cancers. *Science* 254, 1167-73. (1991)
3. zur Hausen, H.: Papillomavirus infections--a major cause of human cancers. *Biochim Biophys Acta* 1288, F55-78. (1996)
4. Ishiji, T.: Molecular mechanism of carcinogenesis by human papillomavirus-16. *J Dermatol* 27, 73-86. (2000)
5. Swan, D. C., S. D. Vernon & J. P. Icenogle: Cellular proteins involved in papillomavirus-induced transformation. *Arch Virol* 138, 105-15 (1994)
6. Munger, K., M. Scheffner, J. M. Huibregtse & P. M. Howley: Interactions of HPV E6 and E7 oncoproteins with tumour suppressor gene products. *Cancer Surv* 12, 197-217 (1992)
7. Havre, P. A., J. Yuan, L. Hedrick, K. R. Cho & P. M. Glazer: p53 inactivation by HPV16 E6 results in increased mutagenesis in human cells. *Cancer Res* 55, 4420-4. (1995)
8. Howley, P. M., K. Munger, H. Romanczuk, M. Scheffner & J. M. Huibregtse: Cellular targets of the oncoproteins encoded by the cancer associated human papillomaviruses. *Princess Takamatsu Symp* 22, 239-48 (1991)
9. Schiffman, M. H. & L. A. Brinton: The epidemiology of cervical carcinogenesis. *Cancer* 76, 1888-901. (1995)
10. Tortolero-Luna, G.: Epidemiology of genital human papillomavirus. *Hematol Oncol Clin North Am* 13, 245-57, x. (1999)
11. Schoell, W. M., M. F. Janicek & R. Mirhashemi: Epidemiology and biology of cervical cancer. *Semin Surg Oncol* 16, 203-11. (1999)
12. Tyring, S. K.: Human papillomavirus infections: epidemiology, pathogenesis, and host immune response. *J Am Acad Dermatol* 43, S18-26. (2000)
13. Ho, G. Y., R. D. Burk, S. Klein, A. S. Kadish, C. J. Chang, P. Palan, J. Basu, R. Tachezy, R. Lewis & S. Romney: Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. *J Natl Cancer Inst* 87, 1365-71. (1995)

14. Ho, G. Y., R. Bierman, L. Beardsley, C. J. Chang & R. D. Burk: Natural history of cervicovaginal papillomavirus infection in young women. *N Engl J Med* 338, 423-8. (1998)
15. Moscicki, A. B., S. Shiboski, J. Broering, K. Powell, L. Clayton, N. Jay, T. M. Darragh, R. Brescia, S. Kanowitz, S. B. Miller, J. Stone, E. Hanson & J. Palefsky: The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. *J Pediatr* 132, 277-84. (1998)
16. Remmink, A. J., J. M. Walboomers, T. J. Helmerhorst, F. J. Voorhorst, L. Rozendaal, E. K. Risse, C. J. Meijer & P. Kenemans: The presence of persistent high-risk HPV genotypes in dysplastic cervical lesions is associated with progressive disease: natural history up to 36 months. *Int J Cancer* 61, 306-11. (1995)
17. Gillison, M. L., W. M. Koch & K. V. Shah: Human papillomavirus in head and neck squamous cell carcinoma: are some head and neck cancers a sexually transmitted disease? *Curr Opin Oncol* 11, 191-9. (1999)
18. Gillison, M. L., W. M. Koch, R. B. Capone, M. Spafford, W. H. Westra, L. Wu, M. L. Zahurak, R. W. Daniel, M. Viglione, D. E. Symer, K. V. Shah & D. Sidransky: Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst* 92, 709-20. (2000)
19. van Houten, V. M., P. J. Snijders, M. W. van den Brekel, J. A. Kummer, C. J. Meijer, B. van Leeuwen, F. Denkers, L. E. Smeele, G. B. Snow & R. H. Brakenhoff: Biological evidence that human papillomaviruses are etiologically involved in a subgroup of head and neck squamous cell carcinomas. *Int J Cancer* 93, 232-5. (2001)
20. Mork, J., A. K. Lie, E. Glattre, G. Hallmans, E. Jellum, P. Koskela, B. Moller, E. Pukkala, J. T. Schiller, L. Youngman, M. Lehtinen & J. Dillner: Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. *N Engl J Med* 344, 1125-31. (2001)
21. Biliris, K. A., E. Koumantakis, D. N. Dokianakis, G. Sourvinos & D. A. Spandidos: Human papillomavirus infection of non-melanoma skin cancers in immunocompetent hosts. *Cancer Lett* 161, 83-8. (2000)
22. Harwood, C. A., T. Surentheran, J. M. McGregor, P. J. Spink, I. M. Leigh, J. Breuer & C. M. Proby: Human papillomavirus infection and non-melanoma skin cancer in immunosuppressed and immunocompetent individuals. *J Med Virol* 61, 289-97. (2000)
23. Harwood, C. A., J. M. McGregor, C. M. Proby & J. Breuer: Human papillomavirus and the development of non-melanoma skin cancer. *J Clin Pathol* 52, 249-53. (1999)
24. Herber, R., A. Liem, H. Pitot & P. F. Lambert: Squamous epithelial hyperplasia and carcinoma in mice transgenic for the human papillomavirus type 16 E7 oncogene. *J Virol* 70, 1873-81. (1996)
25. Kang, J. K., J. H. Kim, S. H. Lee, D. H. Kim, H. S. Kim, J. E. Lee & J. S. Seo: Development of spontaneous hyperplastic skin lesions and chemically induced skin papillomas in transgenic mice expressing human papillomavirus type 16 E6/E7 genes. *Cancer Lett* 160, 177-83. (2000)
26. Eckert, R. L., J. F. Crish, S. Balasubramanian & E. A. Rorke: Transgenic animal models of human papillomavirus-dependent disease (Review). *Int J Oncol* 16, 853-70. (2000)
27. Smetana, Z., T. Keller, S. Leventon-Kriss, M. Huszar, A. Lindner, S. Mitrani-Rosenbaum, E. Mendelson & S. Smetana: Presence of human papilloma virus in transitional cell carcinoma in Jewish population in Israel. *Cell Mol Biol (Noisy-le-grand)* 41, 1017-23. (1995)
28. LaRue, H., M. Simoneau & Y. Fradet: Human papillomavirus in transitional cell carcinoma of the urinary bladder. *Clin Cancer Res* 1, 435-40. (1995)
29. De Gaetani, C., G. Ferrari, E. Righi, S. Bettelli, M. Migaldi, P. Ferrari & G. P. Trentini: Detection of human papillomavirus DNA in urinary bladder carcinoma by in situ hybridisation. *J Clin Pathol* 52, 103-6. (1999)
30. Anwar, K., H. Naiki, K. Nakakuki & M. Inuzuka: High frequency of human papillomavirus infection in carcinoma of the urinary bladder. *Cancer* 70, 1967-73. (1992)
31. Agliano, A. M., A. Gradilone, P. Gazzaniga, M. Napolitano, R. Vercillo, L. Albonici, G. Naso, V. Manzari, L. Frati & A. Vecchione: High frequency of human papillomavirus detection in urinary bladder cancer. *Urol Int* 53, 125-9 (1994)
32. Chetsanga, C., P. U. Malmstrom, U. Gyllensten, J. Moreno-Lopez, Z. Dinter & U. Pettersson: Low incidence of human papillomavirus type 16 DNA in bladder tumor detected by the polymerase chain reaction. *Cancer* 69, 1208-11. (1992)
33. Lopez-Beltran, A. & E. Munoz: Transitional cell carcinoma of the bladder: low incidence of human papillomavirus DNA detected by the polymerase chain reaction and in situ hybridization. *Histopathology* 26, 565-9. (1995)
34. Westenend, P. J., J. A. Stoop & J. G. Hendriks: Human papillomaviruses 6/11, 16/18 and 31/33/51 are not associated with squamous cell carcinoma of the urinary bladder. *BJU Int* 88, 198-201. (2001)
35. Noel, J. C., M. O. Peny, O. Mat, M. Antoine, C. Firket, O. Detremmerie, L. Thiry, A. Verhest & P. Vereerstraeten: Human papillomavirus type 16 associated with multifocal transitional cell carcinomas of the bladder in two transplanted patients. *Transpl Int* 7, 340-3. (1994)
36. Noel, J. C., L. Thiry, A. Verhest, N. Deschepper, M. O. Peny, A. A. Sattar, C. C. Schulman & J. Haot: Transitional cell carcinoma of the bladder: evaluation of the role of human papillomaviruses. *Urology* 44, 671-5. (1994)
37. Miyagi, J., K. Tsuhako, T. Kinjo, T. Iwamasa & T. Hirayasu: Recent striking changes in histological differentiation and rate of human papillomavirus infection in squamous cell carcinoma of the lung in Okinawa, a subtropical island in southern Japan. *J Clin Pathol* 53, 676-84. (2000)
38. Nakazato, I., T. Hirayasu, Y. Kamada, K. Tsuhako & T. Iwamasa: Carcinoma of the lung in Okinawa, Japan: with special reference to squamous cell carcinoma and squamous metaplasia. *Pathol Int* 47, 659-72. (1997)
39. Hirayasu, T., T. Iwamasa, Y. Kamada, Y. Koyanagi, H. Usuda & K. Genka: Human papillomavirus DNA in squamous cell carcinoma of the lung. *J Clin Pathol* 49, 810-7. (1996)

40. Popper, H. H., Y. el-Shabrawi, W. Wockel, G. Hofler, L. Kenner, F. M. Juttner-Smolle & M. G. Pongratz: Prognostic importance of human papilloma virus typing in squamous cell papilloma of the bronchus: comparison of in situ hybridization and the polymerase chain reaction. *Hum Pathol* 25, 1191-7. (1994)
41. Soini, Y., K. Nuorva, D. Kamel, R. Pollanen, K. Vahakangas, V. P. Lehto & P. Paakko: Presence of human papillomavirus DNA and abnormal p53 protein accumulation in lung carcinoma. *Thorax* 51, 887-93. (1996)
42. Szabo, I., R. Sepp, K. Nakamoto, M. Maeda, H. Sakamoto & H. Uda: Human papillomavirus not found in squamous and large cell lung carcinomas by polymerase chain reaction. *Cancer* 73, 2740-4. (1994)
43. Chang, F., S. Syrjanen, Q. Shen, M. Cintorino, R. Santopietro, P. Tosi & K. Syrjanen: Human papillomavirus involvement in esophageal carcinogenesis in the high-incidence area of China. A study of 700 cases by screening and type-specific in situ hybridization. *Scand J Gastroenterol* 35, 123-30. (2000)
44. Chen, L. P., E. K. Thomas, S. L. Hu, I. Hellstrom & K. E. Hellstrom: Human papillomavirus type 16 nucleoprotein E7 is a tumor rejection antigen. *Proc Natl Acad Sci U S A* 88, 110-4. (1991)
45. Gatti, R. A. & R. A. Good: Occurrence of malignancy in immunodeficiency diseases. A literature review. *Cancer* 28, 89-98. (1971)
46. Johnstone, F. D., E. McGoogan, G. E. Smart, R. P. Brettle & R. J. Prescott: A population-based, controlled study of the relation between HIV infection and cervical neoplasia. *Br J Obstet Gynaecol* 101, 986-91. (1994)
47. Penn, I.: Tumor incidence in human allograft recipients. *Transplant Proc* 11, 1047-51. (1979)
48. Schneider, V., S. Kay & H. M. Lee: Immunosuppression as a high-risk factor in the development of condyloma acuminatum and squamous neoplasia of the cervix. *Acta Cytol* 27, 220-4. (1983)
49. Sun, X. W., L. Kuhn, T. V. Ellerbrock, M. A. Chiasson, T. J. Bush & T. C. Wright, Jr.: Human papillomavirus infection in women infected with the human immunodeficiency virus. *N Engl J Med* 337, 1343-9. (1997)
50. Tweddel, G., P. Heller, M. Cunnane, H. Multhaupt & K. Roth: The correlation between HIV seropositivity, cervical dysplasia, and HPV subtypes 6/11, 16/18, 31/33/35. *Gynecol Oncol* 52, 161-4. (1994)
51. Ressing, M. E., W. J. van Driel, E. Celis, A. Sette, M. P. Brandt, M. Hartman, J. D. Anholts, G. M. Schreuder, W. B. ter Harmsel, G. J. Fleuren, B. J. Trimbos, W. M. Kast & C. J. Melief: Occasional memory cytotoxic T-cell responses of patients with human papillomavirus type 16-positive cervical lesions against a human leukocyte antigen-A *0201-restricted E7-encoded epitope. *Cancer Res* 56, 582-8. (1996)
52. Koopman, L. A., W. E. Corver, A. R. van der Slik, M. J. Giphart & G. J. Fleuren: Multiple genetic alterations cause frequent and heterogeneous human histocompatibility leukocyte antigen class I loss in cervical cancer. *J Exp Med* 191, 961-76. (2000)
53. Cromme, F. V., J. Airey, M. T. Heemels, H. L. Ploegh, P. J. Keating, P. L. Stern, C. J. Meijer & J. M. Walboomers: Loss of transporter protein, encoded by the TAP-1 gene, is highly correlated with loss of HLA expression in cervical carcinomas. *J Exp Med* 179, 335-40. (1994)
54. Ressing, M. E., J. H. de Jong, R. M. Brandt, J. W. Drijfhout, W. E. Benckhuijsen, G. M. Schreuder, R. Offringa, W. M. Kast & C. J. Melief: Differential binding of viral peptides to HLA-A2 alleles. Implications for human papillomavirus type 16 E7 peptide-based vaccination against cervical carcinoma. *Eur J Immunol* 29, 1292-303. (1999)
55. Feltkamp, M. C., H. L. Smits, M. P. Vierboom, R. P. Minnaar, B. M. de Jongh, J. W. Drijfhout, J. ter Schegget, C. J. Melief & W. M. Kast: Vaccination with cytotoxic T lymphocyte epitope-containing peptide protects against a tumor induced by human papillomavirus type 16-transformed cells. *Eur J Immunol* 23, 2242-9. (1993)
56. Rudolf, M. P., S. Man, C. J. Melief, A. Sette & W. M. Kast: Human T-cell responses to HLA-A-restricted high binding affinity peptides of human papillomavirus type 18 proteins E6 and E7. *Clin Cancer Res* 7, 788s-95s. (2001)
57. Azoury-Ziadeh, R., K. Herd, G. J. Fernando, I. H. Frazer & R. W. Tindle: T-helper epitopes identified within the E6 transforming protein of cervical cancer-associated human papillomavirus type 16. *Viral Immunol* 12, 297-312 (1999)
58. Garcia, A. M., V. F. Ortiz-Navarrete, M. L. Mora-Garcia, F. Flores-Borja, A. Diaz-Quinonez, A. Isibasi-Araujo, C. Trejo-Becerril, R. Chacon-Salinas, J. Hernandez-Montes, J. Granados-Arreola, C. de Leo & B. Weiss-Steider: Identification of peptides presented by HLA class I molecules on cervical cancer cells with HPV-18 infection. *Immunol Lett* 67, 167-77. (1999)
59. Bauer, S., K. Heeg, H. Wagner & G. B. Lipford: Identification of H-2Kb binding and immunogenic peptides from human papilloma virus tumour antigens E6 and E7. *Scand J Immunol* 42, 317-23. (1995)
60. Feltkamp, M. C., M. P. Vierboom, R. E. Toes, F. Ossendorp, J. ter Schegget, C. J. Melief & W. M. Kast: Competition inhibition of cytotoxic T-lymphocyte (CTL) lysis, a more sensitive method to identify candidate CTL epitopes than induction of antibody-detected MHC class I stabilization. *Immunol Lett* 47, 1-8. (1995)
61. Altmann, A., I. Jochmus-Kudielka, R. Frank, H. Gausepohl, U. Moebius, L. Gissmann & S. C. Meuer: Definition of immunogenic determinants of the human papillomavirus type 16 nucleoprotein E7. *Eur J Cancer* 28, 326-33 (1992)
62. Davies, D. H., C. M. Hill, J. B. Rothbard & B. M. Chain: Definition of murine T helper cell determinants in the major capsid protein of human papillomavirus type 16. *J Gen Virol* 71, 2691-8. (1990)
63. Muderspach, L., S. Wilczynski, L. Roman, L. Bade, J. Felix, L. A. Small, W. M. Kast, G. Fascio, V. Marty & J. Weber: A phase I trial of a human papillomavirus (HPV) peptide vaccine for women with high-grade cervical and vulvar intraepithelial neoplasia who are HPV 16 positive. *Clin Cancer Res* 6, 3406-16. (2000)
64. van Driel, W. J., M. E. Ressing, G. G. Kenter, R. M. Brandt, E. J. Krul, A. B. van Rossum, E. Schuuring, R. Offringa, T. Bauknecht, A. Tamm-Hermelink, P. A. van Dam, G. J. Fleuren, W. M. Kast, C. J. Melief & J. B. Trimbos: Vaccination with HPV16 peptides of patients

with advanced cervical carcinoma: clinical evaluation of a phase I-II trial. *Eur J Cancer* 35, 946-52. (1999)
65. van Elsas, A., R. P. Sutmuller, A. A. Hurwitz, J. Ziskin, J. Villasenor, J. P. Medema, W. W. Overwijk, N. P. Restifo, C. J. Melief, R. Offringa & J. P. Allison: Elucidating the autoimmune and antitumor effector mechanisms of a treatment based on cytotoxic T lymphocyte antigen-4 blockade in combination with a B16 melanoma vaccine: comparison of prophylaxis and therapy. *J Exp Med* 194, 481-9. (2001)
66. Banchereau, J., F. Briere, C. Caux, J. Davoust, S. Lebecque, Y. J. Liu, B. Pulendran & K. Palucka: Immunobiology of dendritic cells. *Annu Rev Immunol* 18, 767-811 (2000)
67. Liu, Y. J., H. Kanzler, V. Soumelis & M. Gilliet: Dendritic cell lineage, plasticity and cross-regulation. *Nat Immunol* 2, 585-9. (2001)
68. Blom, B., S. Ho, S. Antonenko & Y. J. Liu: Generation of interferon alpha-producing predendritic cell (Pre-DC)2 from human CD34(+) hematopoietic stem cells. *J Exp Med* 192, 1785-96. (2000)
69. Liu, Y. J., N. Kadowaki, M. C. Rissoan & V. Soumelis: T cell activation and polarization by DC1 and DC2. *Curr Top Microbiol Immunol* 251, 149-59 (2000)
70. Mohamadzadeh, M., F. Berard, G. Essert, C. Chalouni, B. Pulendran, J. Davoust, G. Bridges, A. K. Palucka & J. Banchereau: Interleukin 15 skews monocyte differentiation into dendritic cells with features of Langerhans cells. *J Exp Med* 194, 1013-20. (2001)
71. Rudolf, M. P., S. C. Fausch, D. M. Da Silva & W. M. Kast: Human dendritic cells are activated by chimeric human papillomavirus type-16 virus-like particles and induce epitope-specific human T cell responses *in vitro*. *J Immunol* 166, 5917-24. (2001)
72. Murakami, M., K. J. Gurski, F. M. Marincola, J. Ackland & M. A. Steller: Induction of specific CD8+ T-lymphocyte responses using a human papillomavirus-16 E6/E7 fusion protein and autologous dendritic cells. *Cancer Res* 59, 1184-7. (1999)
73. DeMatos, P., Z. Abdel-Wahab, C. Vervaert, D. Hester & H. Seigler: Pulsing of dendritic cells with cell lysates from either B16 melanoma or MCA-106 fibrosarcoma yields equally effective vaccines against B16 tumors in mice. *J Surg Oncol* 68, 79-91. (1998)
74. Wolfers, J., A. Lozier, G. Raposo, A. Regnault, C. Thery, C. Masurier, C. Flament, S. Pouzieux, F. Faure, T. Tursz, E. Angevin, S. Amigorena & L. Zitvogel: Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. *Nat Med* 7, 297-303. (2001)
75. Heiser, A., M. A. Maurice, D. R. Yancey, N. Z. Wu, P. Dahm, S. K. Pruitt, D. Boczkowski, S. K. Nair, M. S. Ballo, E. Gilboa & J. Vieweg: Induction of polyclonal prostate cancer-specific CTL using dendritic cells transfected with amplified tumor RNA. *J Immunol* 166, 2953-60. (2001)
76. Bubenik, J.: Genetically engineered dendritic cell-based cancer vaccines (review). *Int J Oncol* 18, 475-8. (2001)
77. Gilboa, E., S. K. Nair & H. K. Lyerly: Immunotherapy of cancer with dendritic-cell-based vaccines. *Cancer Immunol Immunother* 46, 82-7. (1998)
78. Indrova, M., J. Bubenik, J. Simova, V. Vonka, S. Nemeckova, L. Mendoza & M. Reinis: Therapy of HPV 16-associated carcinoma with dendritic cell-based vaccines: *in vitro* priming of the effector cell responses by DC pulsed with tumour lysates and synthetic RAHYNIVTF peptide. *Int J Mol Med* 7, 97-100. (2001)
79. Nestle, F. O., S. Alijagic, M. Gilliet, Y. Sun, S. Grabbe, R. Dummer, G. Burg & D. Schadendorf: Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat Med* 4, 328-32. (1998)
80. Geiger, J. D., R. J. Hutchinson, L. F. Hohenkirk, E. A. McKenna, G. A. Yanik, J. E. Levine, A. E. Chang, T. M. Braun & J. J. Mule: Vaccination Of Pediatric Solid Tumor Patients with Tumor Lysate-pulsed Dendritic Cells Can Expand Specific T Cells and Mediate Tumor Regression. *Cancer Res* 61, 8513-9. (2001)
81. Fields, R. C., K. Shimizu & J. J. Mule: Murine dendritic cells pulsed with whole tumor lysates mediate potent antitumor immune responses *in vitro* and *in vivo*. *Proc Natl Acad Sci U S A* 95, 9482-7. (1998)
82. Paczesny, S., S. Beranger, J. L. Salzmann, D. Klatzmann & B. M. Colombo: Protection of mice against leukemia after vaccination with bone marrow- derived dendritic cells loaded with apoptotic leukemia cells. *Cancer Res* 61, 2386-9. (2001)
83. Zitvogel, L., A. Regnault, A. Lozier, J. Wolfers, C. Flament, D. Tenza, P. Ricciardi-Castagnoli, G. Raposo & S. Amigorena: Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat Med* 4, 594-600. (1998)
84. Ossevoort, M. A., M. C. Feltkamp, K. J. van Veen, C. J. Melief & W. M. Kast: Dendritic cells as carriers for a cytotoxic T-lymphocyte epitope-based peptide vaccine in protection against a human papillomavirus type 16-induced tumor. *J Immunother Emphasis Tumor Immunol* 18, 86-94. (1995)
85. Mayordomo, J. I., T. Zorina, W. J. Storkus, L. Zitvogel, M. D. Garcia-Prats, A. B. DeLeo & M. T. Lotze: Bone marrow-derived dendritic cells serve as potent adjuvants for peptide-based antitumor vaccines. *Stem Cells* 15, 94-103 (1997)
86. Mayordomo, J. I., T. Zorina, W. J. Storkus, L. Zitvogel, C. Celluzzi, L. D. Falo, C. J. Melief, S. T. Ildstad, W. M. Kast, A. B. Deleo & et al.: Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic antitumour immunity. *Nat Med* 1, 1297-302. (1995)
87. Chang, E. Y., C. H. Chen, H. Ji, T. L. Wang, K. Hung, B. P. Lee, A. Y. Huang, R. J. Kurman, D. M. Pardoll & T. Wu: Antigen-specific cancer immunotherapy using a GM-CSF secreting allogeneic tumor cell-based vaccine. *Int J Cancer* 86, 725-30. (2000)
88. Hallez, S., O. Detremmerie, C. Giannouli, K. Thielemans, T. F. Gajewski, A. Burny & O. Leo: Interleukin-12-secreting human papillomavirus type 16-transformed cells provide a potent cancer vaccine that generates E7-directed immunity. *Int J Cancer* 81, 428-37. (1999)
89. Holmes, L. M., J. Li, R. P. Sticca, T. E. Wagner & Y. Wei: A rapid, novel strategy to induce tumor cell-specific cytotoxic T lymphoctye responses using instant dentritomas. *J Immunother* 24, 122-9. (2001)
90. Tanaka, Y., S. Koido, D. Chen, S. J. Gendler, D. Kufe & J. Gong: Vaccination with Allogeneic Dendritic Cells Fused to Carcinoma Cells Induces Antitumor Immunity in

MUC1 Transgenic Mice. *Clin Immunol* 101, 192-200. (2001)
91. Parajuli, P., R. L. Mosley, V. Pisarev, J. Chavez, A. Ulrich, M. Varney, R. K. Singh & J. E. Talmadge: Flt3 ligand and granulocyte-macrophage colony-stimulating factor preferentially expand and stimulate different dendritic and T-cell subsets. *Exp Hematol* 29, 1185-93. (2001)
92. Avigan, D., Z. Wu, J. Gong, R. Joyce, J. Levine, A. Elias, P. Richardson, J. Milano, L. Kennedy, K. Anderson & D. Kufe: Selective *in vivo* mobilization with granulocyte macrophage colony- stimulating factor (GM-CSF)/granulocyte-CSF as compared to G-CSF alone of dendritic cell progenitors from peripheral blood progenitor cells in patients with advanced breast cancer undergoing autologous transplantation. *Clin Cancer Res* 5, 2735-41. (1999)
93. Maraskovsky, E., B. Pulendran, K. Brasel, M. Teepe, E. R. Roux, K. Shortman, S. D. Lyman & H. J. McKenna: Dramatic numerical increase of functionally mature dendritic cells in FLT3 ligand-treated mice. *Adv Exp Med Biol* 417, 33-40 (1997)
94. Chen, K., S. Braun, S. Lyman, Y. Fan, C. M. Traycoff, E. A. Wiebke, J. Gaddy, G. Sledge, H. E. Broxmeyer & K. Cornetta: Antitumor activity and immunotherapeutic properties of Flt3-ligand in a murine breast cancer model. *Cancer Res* 57, 3511-6. (1997)
95. Lynch, D. H., A. Andreasen, E. Maraskovsky, J. Whitmore, R. E. Miller & J. C. Schuh: Flt3 ligand induces tumor regression and antitumor immune responses *in vivo*. *Nat Med* 3, 625-31. (1997)
96. Fernandez, N. C., A. Lozier, C. Flament, P. Ricciardi-Castagnoli, D. Bellet, M. Suter, M. Perricaudet, T. Tursz, E. Maraskovsky & L. Zitvogel: Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses *in vivo*. *Nat Med* 5, 405-11. (1999)
97. Bogen, B.: Peripheral T cell tolerance as a tumor escape mechanism: deletion of CD4+ T cells specific for a monoclonal immunoglobulin idiotype secreted by a plasmacytoma. *Eur J Immunol* 26, 2671-9. (1996)
98. Forster, I., R. Hirose, J. M. Arbeit, B. E. Clausen & D. Hanahan: Limited capacity for tolerization of CD4+ T cells specific for a pancreatic beta cell neo-antigen. *Immunity* 2, 573-85. (1995)
99. Hahne, M., D. Rimoldi, M. Schroter, P. Romero, M. Schreier, L. E. French, P. Schneider, T. Bornand, A. Fontana, D. Lienard, J. Cerottini & J. Tschopp: Melanoma cell expression of Fas(Apo-1/CD95) ligand: implications for tumor immune escape. *Science* 274, 1363-6. (1996)
100. Kurts, C., W. R. Heath, H. Kosaka, J. F. Miller & F. R. Carbone: The peripheral deletion of autoreactive CD8+ T cells induced by cross- presentation of self-antigens involves signaling through CD95 (Fas, Apo- 1). *J Exp Med* 188, 415-20. (1998)
101. Webb, S., C. Morris & J. Sprent: Extrathymic tolerance of mature T cells: clonal elimination as a consequence of immunity. *Cell* 63, 1249-56. (1990)
102. Zeytun, A., M. Hassuneh, M. Nagarkatti & P. S. Nagarkatti: Fas-Fas ligand-based interactions between tumor cells and tumor- specific cytotoxic T lymphocytes: a lethal two-way street. *Blood* 90, 1952-9. (1997)
103. Lo, D., J. Freedman, S. Hesse, R. D. Palmiter, R. L. Brinster & L. A. Sherman: Peripheral tolerance to an islet cell-specific hemagglutinin transgene affects both CD4+ and CD8+ T cells. *Eur J Immunol* 22, 1013-22. (1992)
104. Schonrich, G., U. Kalinke, F. Momburg, M. Malissen, A. M. Schmitt-Verhulst, B. Malissen, G. J. Hammerling & B. Arnold: Down-regulation of T cell receptors on self-reactive T cells as a novel mechanism for extrathymic tolerance induction. *Cell* 65, 293-304. (1991)
105. Mizoguchi, H., J. J. O'Shea, D. L. Longo, C. M. Loeffler, D. W. McVicar & A. C. Ochoa: Alterations in signal transduction molecules in T lymphocytes from tumor-bearing mice. *Science* 258, 1795-8. (1992)
106. Speiser, D. E., R. Miranda, A. Zakarian, M. F. Bachmann, K. McKall-Faienza, B. Odermatt, D. Hanahan, R. M. Zinkernagel & P. S. Ohashi: Self antigens expressed by solid tumors Do not efficiently stimulate naive or activated T cells: implications for immunotherapy. *J Exp Med* 186, 645-53. (1997)
107. Staveley-O'Carroll, K., E. Sotomayor, J. Montgomery, I. Borrello, L. Hwang, S. Fein, D. Pardoll & H. Levitsky: Induction of antigen-specific T cell anergy: An early event in the course of tumor progression. *Proc Natl Acad Sci U S A* 95, 1178-83. (1998)
108. Ohashi, P. S., S. Oehen, K. Buerki, H. Pircher, C. T. Ohashi, B. Odermatt, B. Malissen, R. M. Zinkernagel & H. Hengartner: Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell* 65, 305-17. (1991)
109. Oldstone, M. B., M. Nerenberg, P. Southern, J. Price & H. Lewicki: Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. *Cell* 65, 319-31. (1991)
110. Wick, M., P. Dubey, H. Koeppen, C. T. Siegel, P. E. Fields, L. Chen, J. A. Bluestone & H. Schreiber: Antigenic cancer cells grow progressively in immune hosts without evidence for T cell exhaustion or systemic anergy. *J Exp Med* 186, 229-38. (1997)
111. Johnston, J. V., A. R. Malacko, M. T. Mizuno, P. McGowan, I. Hellstrom, K. E. Hellstrom, H. Marquardt & L. Chen: B7-CD28 costimulation unveils the hierarchy of tumor epitopes recognized by major histocompatibility complex class I-restricted CD8+ cytolytic T lymphocytes. *J Exp Med* 183, 791-800. (1996)
112. Kurts, C., R. M. Sutherland, G. Davey, M. Li, A. M. Lew, E. Blanas, F. R. Carbone, J. F. Miller & W. R. Heath: CD8 T cell ignorance or tolerance to islet antigens depends on antigen dose. *Proc Natl Acad Sci U S A* 96, 12703-7. (1999)
113. Kurts, C., F. R. Carbone, M. Barnden, E. Blanas, J. Allison, W. R. Heath & J. F. Miller: CD4+ T cell help impairs CD8+ T cell deletion induced by cross- presentation of self-antigens and favors autoimmunity. *J Exp Med* 186, 2057-62. (1997)
114. Jenkins, M. K. & R. H. Schwartz: Antigen presentation by chemically modified splenocytes induces antigen- specific T cell unresponsiveness *in vitro* and *in vivo*. *J Exp Med* 165, 302-19. (1987)
115. Quill, H. & R. H. Schwartz: Stimulation of normal inducer T cell clones with antigen presented by purified Ia molecules in planar lipid membranes: specific induction of a long-lived state of proliferative nonresponsiveness. *J Immunol* 138, 3704-12. (1987)

116. Schwartz, R. H.: A cell culture model for T lymphocyte clonal anergy. *Science* 248, 1349-56. (1990)
117. Deeths, M. J., R. M. Kedl & M. F. Mescher: CD8+ T cells become nonresponsive (anergic) following activation in the presence of costimulation. *J Immunol* 163, 102-10. (1999)
118. Tham, E. L. & M. F. Mescher: Signaling alterations in activation-induced nonresponsive cd8 t cells. *J Immunol* 167, 2040-8. (2001)
119. Shrikant, P. & M. F. Mescher: Control of syngeneic tumor growth by activation of CD8+ T cells: efficacy is limited by migration away from the site and induction of nonresponsiveness. *J Immunol* 162, 2858-66. (1999)
120. Shrikant, P., A. Khoruts & M. F. Mescher: CTLA-4 blockade reverses CD8+ T cell tolerance to tumor by a CD4+ T cell- and IL-2-dependent mechanism. *Immunity* 11, 483-93. (1999)
121. Otten, G. R. & R. N. Germain: Split anergy in a CD8+ T cell: receptor-dependent cytolysis in the absence of interleukin-2 production. *Science* 251, 1228-31. (1991)
122. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh & M. Toda: Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 155, 1151-64. (1995)
123. Asano, M., M. Toda, N. Sakaguchi & S. Sakaguchi: Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J Exp Med* 184, 387-96. (1996)
124. Shevach, E. M.: Certified professionals: CD4(+)CD25(+) suppressor T cells. *J Exp Med* 193, F41-6. (2001)
125. Shimizu, J., S. Yamazaki & S. Sakaguchi: Induction of tumor immunity by removing CD25+CD4+ T cells: a common basis between tumor immunity and autoimmunity. *J Immunol* 163, 5211-8. (1999)
126. Harlan, D. M., H. Hengartner, M. L. Huang, Y. H. Kang, R. Abe, R. W. Moreadith, H. Pircher, G. S. Gray, P. S. Ohashi, G. J. Freeman & et al.: Mice expressing both B7-1 and viral glycoprotein on pancreatic beta cells along with glycoprotein-specific transgenic T cells develop diabetes due to a breakdown of T-lymphocyte unresponsiveness. *Proc Natl Acad Sci U S A* 91, 3137-41. (1994)
127. Guerder, S., D. E. Picarella, P. S. Linsley & R. A. Flavell: Costimulator B7-1 confers antigen-presenting-cell function to parenchymal tissue and in conjunction with tumor necrosis factor alpha leads to autoimmunity in transgenic mice. *Proc Natl Acad Sci U S A* 91, 5138-42. (1994)
128. Ganss, R. & D. Hanahan: Tumor microenvironment can restrict the effectiveness of activated antitumor lymphocytes. *Cancer Res* 58, 4673-81. (1998)
129. Melero, I., M. C. Singhal, P. McGowan, H. S. Haugen, J. Blake, K. E. Hellstrom, G. Yang, C. H. Clegg & L. Chen: Immunological ignorance of an E7-encoded cytolytic T-lymphocyte epitope in transgenic mice expressing the E7 and E6 oncogenes of human papillomavirus type 16. *J Virol* 71, 3998-4004. (1997)
130. Sarma, S., Y. Guo, Y. Guilloux, C. Lee, X. F. Bai & Y. Liu: Cytotoxic T lymphocytes to an unmutated tumor rejection antigen P1A: normal development but restrained effector function *in vivo*. *J Exp Med* 189, 811-20. (1999)
131. Morgan, D. J., H. T. Kreuwel & L. A. Sherman: Antigen concentration and precursor frequency determine the rate of CD8+ T cell tolerance to peripherally expressed antigens. *J Immunol* 163, 723-7. (1999)
132. Kurts, C., J. F. Miller, R. M. Subramaniam, F. R. Carbone & W. R. Heath: Major histocompatibility complex class I-restricted cross-presentation is biased towards high dose antigens and those released during cellular destruction. *J Exp Med* 188, 409-14. (1998)
133. Wolkers, M. C., G. Stoetter, F. A. Vyth-Dreese & T. N. Schumacher: Redundancy of direct priming and cross-priming in tumor-specific cd8(+) t cell responses. *J Immunol* 167, 3577-84. (2001)
134. Bai, X. F., J. X. Gao, J. Liu, J. Wen, P. Zheng & Y. Liu: On the site and mode of antigen presentation for the initiation of clonal expansion of CD8 T cells specific for a natural tumor antigen. *Cancer Res* 61, 6860-7. (2001)
135. Ochsenbein, A. F., P. Klenerman, U. Karrer, B. Ludewig, M. Pericin, H. Hengartner & R. M. Zinkernagel: Immune surveillance against a solid tumor fails because of immunological ignorance. *Proc Natl Acad Sci U S A* 96, 2233-8. (1999)
136. Ochsenbein, A. F., S. Sierro, B. Odermatt, M. Pericin, U. Karrer, J. Hermans, S. Hemmi, H. Hengartner & R. M. Zinkernagel: Roles of tumour localization, second signals and cross priming in cytotoxic T-cell induction. *Nature* 411, 1058-64. (2001)
137. Kim, J. A., B. J. Averbook, K. Chambers, K. Rothchild, J. Kjaergaard, R. Papay & S. Shu: Divergent effects of 4-1BB antibodies on antitumor immunity and on tumor-reactive T-cell generation. *Cancer Res* 61, 2031-7. (2001)
138. Lakkis, F. G., A. Arakelov, B. T. Konieczny & Y. Inoue: Immunologic 'ignorance' of vascularized organ transplants in the absence of secondary lymphoid tissue. *Nat Med* 6, 686-8. (2000)
139. Fuchs, E. J. & P. Matzinger: Is cancer dangerous to the immune system? *Semin Immunol* 8, 271-80. (1996)
140. Matzinger, P.: An innate sense of danger. *Semin Immunol* 10, 399-415. (1998)
141. Gorelik, L. & R. A. Flavell: Immune-mediated eradication of tumors through the blockade of transforming growth factor-beta signaling in T cells. *Nat Med* 7, 1118-22. (2001)
142. Shah, A. H. & C. Lee: TGF-beta-based immunotherapy for cancer: breaching the tumor firewall. *Prostate* 45, 167-72. (2000)
143. Larcher, F., R. Murillas, M. Bolontrade, C. J. Conti & J. L. Jorcano: VEGF/VPF overexpression in skin of transgenic mice induces angiogenesis, vascular hyperpermeability and accelerated tumor development. *Oncogene* 17, 303-11. (1998)
144. Gabrilovich, D. I., H. L. Chen, K. R. Girgis, H. T. Cunningham, G. M. Meny, S. Nadaf, D. Kavanaugh & D. P. Carbone: Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. *Nat Med* 2, 1096-103. (1996)
145. Gunzer, M., A. Schafer, S. Borgmann, S. Grabbe, K. S. Zanker, E. B. Brocker, E. Kampgen & P. Friedl: Antigen presentation in extracellular matrix: interactions of T cells

with dendritic cells are dynamic, short lived, and sequential. *Immunity* 13, 323-32. (2000)
146. Gretz, J. E., A. O. Anderson & S. Shaw: Cords, channels, corridors and conduits: critical architectural elements facilitating cell interactions in the lymph node cortex. *Immunol Rev* 156, 11-24. (1997)
147. Bromley, S. K., D. A. Peterson, M. D. Gunn & M. L. Dustin: Cutting edge: hierarchy of chemokine receptor and TCR signals regulating T cell migration and proliferation. *J Immunol* 165, 15-9. (2000)
148. Liu, X., X. F. Bai, J. Wen, J. X. Gao, J. Liu, P. Lu, Y. Wang, P. Zheng & Y. Liu: B7H Costimulates Clonal Expansion of, and Cognate Destruction of Tumor Cells by, CD8(+) T Lymphocytes *In vivo*. *J Exp Med* 194, 1339-48. (2001)
149. Bai, X. F., J. Bender, J. Liu, H. Zhang, Y. Wang, O. Li, P. Du, P. Zheng & Y. Liu: Local costimulation reinvigorates tumor-specific cytolytic t lymphocytes for experimental therapy in mice with large tumor burdens. *J Immunol* 167, 3936-43. (2001)
150. Allison, J., L. A. Stephens, T. W. Kay, C. Kurts, W. R. Heath, J. F. Miller & M. F. Krummel: The threshold for autoimmune T cell killing is influenced by B7-1. *Eur J Immunol* 28, 949-60. (1998)
151. Hallahan, D., J. Kuchibhotla & C. Wyble: Cell adhesion molecules mediate radiation-induced leukocyte adhesion to the vascular endothelium. *Cancer Res* 56, 5150-5. (1996)
152. Quarmby, S., R. D. Hunter & S. Kumar: Irradiation induced expression of CD31, ICAM-1 and VCAM-1 in human microvascular endothelial cells. *Anticancer Res* 20, 3375-81. (2000)
153. Quarmby, S., P. Kumar & S. Kumar: Radiation-induced normal tissue injury: role of adhesion molecules in leukocyte-endothelial cell interactions. *Int J Cancer* 82, 385-95. (1999)
154. Wu, N. Z., B. Klitzman, R. Dodge & M. W. Dewhirst: Diminished leukocyte-endothelium interaction in tumor microvessels. *Cancer Res* 52, 4265-8. (1992)
155. Melder, R. J., G. C. Koenig, B. P. Witwer, N. Safabakhsh, L. L. Munn & R. K. Jain: During angiogenesis, vascular endothelial growth factor and basic fibroblast growth factor regulate natural killer cell adhesion to tumor endothelium. *Nat Med* 2, 992-7. (1996)
156. Heath, W. R., F. Karamalis, J. Donoghue & J. F. Miller: Autoimmunity caused by ignorant CD8+ T cells is transient and depends on avidity. *J Immunol* 155, 2339-49. (1995)
157. Liu, G. Y., P. J. Fairchild, R. M. Smith, J. R. Prowle, D. Kioussis & D. C. Wraith: Low avidity recognition of self-antigen by T cells permits escape from central tolerance. *Immunity* 3, 407-15. (1995)
158. Doan, T., M. Chambers, M. Street, G. J. Fernando, K. Herd, P. Lambert & R. Tindle: Mice expressing the E7 oncogene of HPV16 in epithelium show central tolerance, and evidence of peripheral anergising tolerance, to E7-encoded cytotoxic T-lymphocyte epitopes. *Virology* 244, 352-64. (1998)
159. Doan, T., K. Herd, M. Street, G. Bryson, G. Fernando, P. Lambert & R. Tindle: Human papillomavirus type 16 E7 oncoprotein expressed in peripheral epithelium tolerizes E7-directed cytotoxic T-lymphocyte precursors restricted through human (and mouse) major histocompatibility complex class I alleles. *J Virol* 73, 6166-70. (1999)
160. Frazer, I. H., R. D. Kluyver, G. R. Leggatt, H. Yang Guo, L. Dunn, O. White, C. Harris, A. Liem & P. Lambert: Tolerance or immunity to a tumor antigen expressed in somatic cells can be determined by systemic proinflammatory signals at the time of first antigen exposure. *J Immunol* 167, 6180-7. (2001)
161. Frazer, I. H., D. M. Leippe, L. A. Dunn, A. Liem, R. W. Tindle, G. J. Fernando, W. C. Phelps & P. F. Lambert: Immunological responses in human papillomavirus 16 E6/E7-transgenic mice to E7 protein correlate with the presence of skin disease. *Cancer Res* 55, 2635-9. (1995)
162. Azoury-Ziadeh, R., K. Herd, G. J. Fernando, P. Lambert, I. H. Frazer & R. W. Tindle: Low level expression of human papillomavirus type 16 (HPV16) E6 in squamous epithelium does not elicit E6 specific B- or T-helper immunological responses, or influence the outcome of immunisation with E6 protein. *Virus Res* 73, 189-99. (2001)
163. Hilditch-Maguire, P. A., D. M. Lieppe, D. West, P. F. Lambert & I. H. Frazer: T cell-mediated and non-specific inflammatory mechanisms contribute to the skin pathology of HPV 16 E6E7 transgenic mice. *Intervirology* 42, 43-50 (1999)
164. Dunn, L. A., M. Evander, R. W. Tindle, A. L. Bulloch, R. L. de Kluyver, G. J. Fernando, P. F. Lambert & I. H. Frazer: Presentation of the HPV16E7 protein by skin grafts is insufficient to allow graft rejection in an E7-primed animal. *Virology* 235, 94-103. (1997)
165. Frazer, I. H., G. J. Fernando, N. Fowler, G. R. Leggatt, P. F. Lambert, A. Liem, K. Malcolm & R. W. Tindle: Split tolerance to a viral antigen expressed in thymic epithelium and keratinocytes. *Eur J Immunol* 28, 2791-800. (1998)
166. Borchers, A., J. Braspenning, J. Meijer, W. Osen, L. Gissmann & I. Jochmus: E7-specific cytotoxic T cell tolerance in HPV-transgenic mice. *Arch Virol* 144, 1539-56 (1999)
167. Nakagawa, M., D. P. Stites, S. Farhat, J. R. Sisler, B. Moss, F. Kong, A. B. Moscicki & J. M. Palefsky: Cytotoxic T lymphocyte responses to E6 and E7 proteins of human papillomavirus type 16: relationship to cervical intraepithelial neoplasia. *J Infect Dis* 175, 927-31. (1997)
168. de Gruijl, T. D., H. J. Bontkes, M. J. Stukart, J. M. Walboomers, A. J. Remmink, R. H. Verheijen, T. J. Helmerhorst, C. J. Meijer & R. J. Scheper: T cell proliferative responses against human papillomavirus type 16 E7 oncoprotein are most prominent in cervical intraepithelial neoplasia patients with a persistent viral infection. *J Gen Virol* 77, 2183-91. (1996)
169. Shepherd, P. S., A. J. Rowe, J. C. Cridland, T. Coletart, P. Wilson & J. C. Luxton: Proliferative T cell responses to human papillomavirus type 16 L1 peptides in patients with cervical dysplasia. *J Gen Virol* 77, 593-602. (1996)
170. de Gruijl, T. D., H. J. Bontkes, J. M. Walboomers, M. J. Stukart, F. S. Doekhie, A. J. Remmink, T. J. Helmerhorst, R. H. Verheijen, M. F. Duggan-Keen, P. L. Stern, C. J. Meijer & R. J. Scheper: Differential T helper cell responses to human papillomavirus type 16 E7 related to viral clearance or persistence in patients with cervical neoplasia: a longitudinal study. *Cancer Res* 58, 1700-6. (1998)

171. Bontkes, H. J., T. D. de Gruijl, A. Bijl, R. H. Verheijen, C. J. Meijer, R. J. Scheper, P. L. Stern, J. E. Burns, N. J. Maitland & J. M. Walboomers: Human papillomavirus type 16 E2-specific T-helper lymphocyte responses in patients with cervical intraepithelial neoplasia. *J Gen Virol* 80, 2453-9. (1999)
172. Kadish, A. S., G. Y. Ho, R. D. Burk, Y. Wang, S. L. Romney, R. Ledwidge & R. H. Angeletti: Lymphoproliferative responses to human papillomavirus (HPV) type 16 proteins E6 and E7: outcome of HPV infection and associated neoplasia. *J Natl Cancer Inst* 89, 1285-93. (1997)
173. Nimako, M., A. N. Fiander, G. W. Wilkinson, L. K. Borysiewicz & S. Man: Human papillomavirus-specific cytotoxic T lymphocytes in patients with cervical intraepithelial neoplasia grade III. *Cancer Res* 57, 4855-61. (1997)
174. Evans, E. M., S. Man, A. S. Evans & L. K. Borysiewicz: Infiltration of cervical cancer tissue with human papillomavirus- specific cytotoxic T-lymphocytes. *Cancer Res* 57, 2943-50. (1997)
175. Barnard, P. & N. A. McMillan: The human papillomavirus E7 oncoprotein abrogates signaling mediated by interferon-alpha. *Virology* 259, 305-13. (1999)
176. Lee, S. J., Y. S. Cho, M. C. Cho, J. H. Shim, K. A. Lee, K. K. Ko, Y. K. Choe, S. N. Park, T. Hoshino, S. Kim, C. A. Dinarello & D. Y. Yoon: Both E6 and E7 oncoproteins of human papillomavirus 16 inhibit IL-18-induced IFN-gamma production in human peripheral blood mononuclear and NK cells. *J Immunol* 167, 497-504. (2001)
177. Le Buanec, H., A. Lachgar, R. D'Anna, J. F. Zagury, B. Bizzini, J. Bernard, D. Ittele, S. Hallez, C. Giannouli, A. Burny & D. Zagury: Induction of cellular immunosuppression by the human papillomavirus type 16 E7 oncogenic protein. *Biomed Pharmacother* 53, 323-8. (1999)
178. Lopez-Ocejo, O., A. Viloria-Petit, M. Bequet-Romero, D. Mukhopadhyay, J. Rak & R. S. Kerbel: Oncogenes and tumor angiogenesis: the HPV-16 E6 oncoprotein activates the vascular endothelial growth factor (VEGF) gene promoter in a p53 independent manner. *Oncogene* 19, 4611-20. (2000)
179. Le Buanec, H., R. D'Anna, A. Lachgar, J. F. Zagury, J. Bernard, D. Ittele, P. d'Alessio, S. Hallez, C. Giannouli, A. Burny, B. Bizzini, R. C. Gallo & D. Zagury: HPV-16 E7 but not E6 oncogenic protein triggers both cellular immunosuppression and angiogenic processes. *Biomed Pharmacother* 53, 424-31. (1999)
180. Loncaster, J. A., R. A. Cooper, J. P. Logue, S. E. Davidson, R. D. Hunter & C. M. West: Vascular endothelial growth factor (VEGF) expression is a prognostic factor for radiotherapy outcome in advanced carcinoma of the cervix. *Br J Cancer* 83, 620-5. (2000)
181. Nestle, F. O. & G. Burg: Dendritic cells: role in skin diseases and therapeutic applications. *Clin Exp Dermatol* 24, 204-7. (1999)
182. Miyagi, J., T. Kinjo, K. Tsuhako, M. Higa, T. Iwamasa, Y. Kamada & T. Hirayasu: Extremely high Langerhans cell infiltration contributes to the favourable prognosis of HPV-infected squamous cell carcinoma and adenocarcinoma of the lung. *Histopathology* 38, 355-67. (2001)
183. Almand, B., J. R. Resser, B. Lindman, S. Nadaf, J. I. Clark, E. D. Kwon, D. P. Carbone & D. I. Gabrilovich: Clinical significance of defective dendritic cell differentiation in cancer. *Clin Cancer Res* 6, 1755-66. (2000)
184. Coppola, D., L. Fu, S. V. Nicosia, S. Kounelis & M. Jones: Prognostic significance of p53, bcl-2, vimentin, and S100 protein- positive Langerhans cells in endometrial carcinoma. *Hum Pathol* 29, 455-62. (1998)
185. Connor, J. P., K. Ferrer, J. P. Kane & J. M. Goldberg: Evaluation of Langerhans' cells in the cervical epithelium of women with cervical intraepithelial neoplasia. *Gynecol Oncol* 75, 130-5. (1999)
186. Nestle, F. O., G. Burg, J. Fah, T. Wrone-Smith & B. J. Nickoloff: Human sunlight-induced basal-cell-carcinoma-associated dendritic cells are deficient in T cell co-stimulatory molecules and are impaired as antigen-presenting cells. *Am J Pathol* 150, 641-51. (1997)
187. Mota, F., N. Rayment, S. Chong, A. Singer & B. Chain: The antigen-presenting environment in normal and human papillomavirus (HPV)-related premalignant cervical epithelium. *Clin Exp Immunol* 116, 33-40. (1999)
188. Steinbrink, K., H. Jonuleit, G. Muller, G. Schuler, J. Knop & A. H. Enk: Interleukin-10-treated human dendritic cells induce a melanoma-antigen- specific anergy in CD8(+) T cells resulting in a failure to lyse tumor cells. *Blood* 93, 1634-42. (1999)
189. Enk, A. H., H. Jonuleit, J. Saloga & J. Knop: Dendritic cells as mediators of tumor-induced tolerance in metastatic melanoma. *Int J Cancer* 73, 309-16. (1997)
190. Katsenelson, N. S., G. V. Shurin, S. N. Bykovskaia, J. Shogan & M. R. Shurin: Human small cell lung carcinoma and carcinoid tumor regulate dendritic cell maturation and function. *Mod Pathol* 14, 40-5. (2001)
191. Moscicki, A. B., N. Hills, S. Shiboski, K. Powell, N. Jay, E. Hanson, S. Miller, L. Clayton, S. Farhat, J. Broering, T. Darragh & J. Palefsky: Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. *Jama* 285, 2995-3002. (2001)
192. Poppe, W. A., P. S. Ide, M. P. Drijkoningen, J. M. Lauweryns & F. A. Van Assche: Tobacco smoking impairs the local immunosurveillance in the uterine cervix. An immunohistochemical study. *Gynecol Obstet Invest* 39, 34-8 (1995)
193. de Gruijl, T. D., H. J. Bontkes, J. M. Walboomers, J. T. Schiller, M. J. Stukart, B. S. Groot, M. M. Chabaud, A. J. Remmink, R. H. Verheijen, T. J. Helmerhorst, C. J. Meijer & R. J. Scheper: Immunoglobulin G responses against human papillomavirus type 16 virus- like particles in a prospective nonintervention cohort study of women with cervical intraepithelial neoplasia. *J Natl Cancer Inst* 89, 630-8. (1997)
194. Qin, Z., G. Richter, T. Schuler, S. Ibe, X. Cao & T. Blankenstein: B cells inhibit induction of T cell-dependent tumor immunity. *Nat Med* 4, 627-30. (1998)
195. Bennett, S. R., F. R. Carbone, T. Toy, J. F. Miller & W. R. Heath: B cells directly tolerize CD8(+) T cells. *J Exp Med* 188, 1977-83. (1998)

196. Scott, M., D. P. Stites & A. B. Moscicki: Th1 cytokine patterns in cervical human papillomavirus infection. *Clin Diagn Lab Immunol* 6, 751-5. (1999)
197. al-Saleh, W., S. L. Giannini, N. Jacobs, M. Moutschen, J. Doyen, J. Boniver & P. Delvenne: Correlation of T-helper secretory differentiation and types of antigen- presenting cells in squamous intraepithelial lesions of the uterine cervix. *J Pathol* 184, 283-90. (1998)
198. Sheu, B. C., R. H. Lin, H. C. Lien, H. N. Ho, S. M. Hsu & S. C. Huang: Predominant th2/tc2 polarity of tumor-infiltrating lymphocytes in human cervical cancer. *J Immunol* 167, 2972-8. (2001)
199. Toes, R. E., E. I. van der Voort, S. P. Schoenberger, J. W. Drijfhout, L. van Bloois, G. Storm, W. M. Kast, R. Offringa & C. J. Melief: Enhancement of tumor outgrowth through CTL tolerization after peptide vaccination is avoided by peptide presentation on dendritic cells. *J Immunol* 160, 4449-56. (1998)
200. Toes, R. E., R. J. Blom, R. Offringa, W. M. Kast & C. J. Melief: Enhanced tumor outgrowth after peptide vaccination. Functional deletion of tumor-specific CTL induced by peptide vaccination can lead to the inability to reject tumors. *J Immunol* 156, 3911-8. (1996)
201. Greenstone, H. L., J. D. Nieland, K. E. de Visser, M. L. De Bruijn, R. Kirnbauer, R. B. Roden, D. R. Lowy, W. M. Kast & J. T. Schiller: Chimeric papillomavirus virus-like particles elicit antitumor immunity against the E7 oncoprotein in an HPV16 tumor model. *Proc Natl Acad Sci U S A* 95, 1800-5. (1998)
202. Bosch, F. X., M. M. Manos, N. Munoz, M. Sherman, A. M. Jansen, J. Peto, M. H. Schiffman, V. Moreno, R. Kurman & K. V. Shah: Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. *J Natl Cancer Inst* 87, 796-802. (1995)
203. Nieland, J. D., D. M. Da Silva, M. P. Velders, K. E. de Visser, J. T. Schiller, M. Muller & W. M. Kast: Chimeric papillomavirus virus-like particles induce a murine self-antigen-specific protective and therapeutic antitumor immune response. *J Cell Biochem* 73, 145-52. (1999)
204. Lenz, P., P. M. Day, Y. Y. Pang, S. A. Frye, P. N. Jensen, D. R. Lowy & J. T. Schiller: Papillomavirus-like particles induce acute activation of dendritic cells. *J Immunol* 166, 5346-55. (2001)
205. Heath, W. R. & F. R. Carbone: Cross-presentation, dendritic cells, tolerance and immunity. *Annu Rev Immunol* 19, 47-64 (2001)
206. Steinman, R. M., S. Turley, I. Mellman & K. Inaba: The induction of tolerance by dendritic cells that have captured apoptotic cells. *J Exp Med* 191, 411-6. (2000)
207. Huang, F. P., N. Platt, M. Wykes, J. R. Major, T. J. Powell, C. D. Jenkins & G. G. MacPherson: A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. *J Exp Med* 191, 435-44. (2000)
208. Roncarolo, M. G., M. K. Levings & C. Traversari: Differentiation of T regulatory cells by immature dendritic cells. *J Exp Med* 193, F5-9. (2001)
209. Hawiger, D., K. Inaba, Y. Dorsett, M. Guo, K. Mahnke, M. Rivera, J. V. Ravetch, R. M. Steinman & M. C. Nussenzweig: Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions *in vivo*. *J Exp Med* 194, 769-79. (2001)
210. Kuwana, M., J. Kaburaki, T. M. Wright, Y. Kawakami & Y. Ikeda: Induction of antigen-specific human CD4(+) T cell anergy by peripheral blood DC2 precursors. *Eur J Immunol* 31, 2547-57. (2001)
211. Brocker, T., M. Riedinger & K. Karjalainen: Targeted expression of major histocompatibility complex (MHC) class II molecules demonstrates that dendritic cells can induce negative but not positive selection of thymocytes *in vivo*. *J Exp Med* 185, 541-50. (1997)
212. Ruedl, C. & M. F. Bachmann: CTL priming by CD8(+) and CD8(-) dendritic cells *in vivo*. *Eur J Immunol* 29, 3762-7. (1999)
213. Melcher, A., S. Todryk, A. Bateman, H. Chong, N. R. Lemoine & R. G. Vile: Adoptive transfer of immature dendritic cells with autologous or allogeneic tumor cells generates systemic antitumor immunity. *Cancer Res* 59, 2802-5. (1999)
214. Todryk, S., A. A. Melcher, N. Hardwick, E. Linardakis, A. Bateman, M. P. Colombo, A. Stoppacciaro & R. G. Vile: Heat shock protein 70 induced during tumor cell killing induces Th1 cytokines and targets immature dendritic cell precursors to enhance antigen uptake. *J Immunol* 163, 1398-408. (1999)
215. Sauter, B., M. L. Albert, L. Francisco, M. Larsson, S. Somersan & N. Bhardwaj: Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. *J Exp Med* 191, 423-34. (2000)
216. Gallucci, S., M. Lolkema & P. Matzinger: Natural adjuvants: endogenous activators of dendritic cells. *Nat Med* 5, 1249-55. java/Propub/medicine/nm199_.fulltext java/Propub/medicine/nm199_.abstract (1999)
217. Gallucci, S. & P. Matzinger: Danger signals: SOS to the immune system. *Curr Opin Immunol* 13, 114-9. (2001)
218. Anderson, C. C., J. M. Carroll, S. Gallucci, J. P. Ridge, A. W. Cheever & P. Matzinger: Testing time-, ignorance-, and danger-based models of tolerance. *J Immunol* 166, 3663-71. (2001)
219. Schultze, J. L., S. Michalak, M. J. Seamon, G. Dranoff, K. Jung, J. Daley, J. C. Delgado, J. G. Gribben & L. M. Nadler: CD40-activated human B cells: an alternative source of highly efficient antigen presenting cells to generate autologous antigen-specific T cells for adoptive immunotherapy. *J Clin Invest* 100, 2757-65. (1997)
220. Vonderheide, R. H., M. O. Butler, J. F. Liu, T. E. Battle, N. Hirano, J. G. Gribben, D. A. Frank, J. L. Schultze & L. M. Nadler: CD40 activation of carcinoma cells increases expression of adhesion and major histocompatibility molecules but fails to induce either CD80/CD86 expression or T cell alloreactivity. *Int J Oncol* 19, 791-8. (2001)
221. Todryk, S. M., A. L. Tutt, M. H. Green, J. A. Smallwood, N. Halanek, A. G. Dalgleish & M. J. Glennie: CD40 ligation for immunotherapy of solid tumours. *J Immunol Methods* 248, 139-47. (2001)
222. Ito, D., K. Ogasawara, K. Iwabuchi, Y. Inuyama & K. Onoe: Induction of CTL responses by simultaneous administration of liposomal peptide vaccine with anti-

CD40 and anti-CTLA-4 mAb. *J Immunol* 164, 1230-5. (2000)
223. French, R. R., H. T. Chan, A. L. Tutt & M. J. Glennie: CD40 antibody evokes a cytotoxic T-cell response that eradicates lymphoma and bypasses T-cell help. *Nat Med* 5, 548-53. (1999)
224. Weinberg, A. D., M. M. Rivera, R. Prell, A. Morris, T. Ramstad, J. T. Vetto, W. J. Urba, G. Alvord, C. Bunce & J. Shields: Engagement of the OX-40 receptor *in vivo* enhances antitumor immunity. *J Immunol* 164, 2160-9. (2000)
225. Vetto, J. T., S. Lum, A. Morris, M. Sicotte, J. Davis, M. Lemon & A. Weinberg: Presence of the T-cell activation marker OX-40 on tumor infiltrating lymphocytes and draining lymph node cells from patients with melanoma and head and neck cancers. *Am J Surg* 174, 258-65. (1997)
226. Hurwitz, A. A., B. A. Foster, E. D. Kwon, T. Truong, E. M. Choi, N. M. Greenberg, M. B. Burg & J. P. Allison: Combination immunotherapy of primary prostate cancer in a transgenic mouse model using CTLA-4 blockade. *Cancer Res* 60, 2444-8. (2000)
227. van Elsas, A., A. A. Hurwitz & J. P. Allison: Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. *J Exp Med* 190, 355-66. (1999)
228. Mokyr, M. B., T. Kalinichenko, L. Gorelik & J. A. Bluestone: Realization of the therapeutic potential of CTLA-4 blockade in low-dose chemotherapy-treated tumor-bearing mice. *Cancer Res* 58, 5301-4. (1998)
229. Hurwitz, A. A., T. F. Yu, D. R. Leach & J. P. Allison: CTLA-4 blockade synergizes with tumor-derived granulocyte-macrophage colony-stimulating factor for treatment of an experimental mammary carcinoma. *Proc Natl Acad Sci U S A* 95, 10067-71. (1998)
230. Yang, Y. F., J. P. Zou, J. Mu, R. Wijesuriya, S. Ono, T. Walunas, J. Bluestone, H. Fujiwara & T. Hamaoka: Enhanced induction of antitumor T-cell responses by cytotoxic T lymphocyte-associated molecule-4 blockade: the effect is manifested only at the restricted tumor-bearing stages. *Cancer Res* 57, 4036-41. (1997)
231. Boussaud, V., P. Soler, J. Moreau, R. G. Goodwin & A. J. Hance: Expression of three members of the TNF-R family of receptors (4-1BB, lymphotoxin-beta receptor, and Fas) in human lung. *Eur Respir J* 12, 926-31. (1998)
232. Broll, K., G. Richter, S. Pauly, F. Hofstaedter & H. Schwarz: CD137 expression in tumor vessel walls. High correlation with malignant tumors. *Am J Clin Pathol* 115, 543-9. (2001)
233. Garni-Wagner, B. A., Z. H. Lee, Y. J. Kim, C. Wilde, C. Y. Kang & B. S. Kwon: 4-1BB is expressed on CD45RAhiROhi transitional T cell in humans. *Cell Immunol* 169, 91-8. (1996)
234. Heinisch, I. V., C. Bizer, W. Volgger & H. U. Simon: Functional CD137 receptors are expressed by eosinophils from patients with IgE-mediated allergic responses but not by eosinophils from patients with non-IgE-mediated eosinophilic disorders. *J Allergy Clin Immunol* 108, 21-8. (2001)
235. Kienzle, G. & J. von Kempis: CD137 (ILA/4-1BB), expressed by primary human monocytes, induces monocyte activation and apoptosis of B lymphocytes. *Int Immunol* 12, 73-82. (2000)
236. Melero, I., J. V. Johnston, W. W. Shufford, R. S. Mittler & L. Chen: NK1.1 cells express 4-1BB (CDw137) costimulatory molecule and are required for tumor immunity elicited by anti-4-1BB monoclonal antibodies. *Cell Immunol* 190, 167-72. (1998)
237. Pollok, K. E., Y. J. Kim, Z. Zhou, J. Hurtado, K. K. Kim, R. T. Pickard & B. S. Kwon: Inducible T cell antigen 4-1BB. Analysis of expression and function. *J Immunol* 150, 771-81. (1993)
238. Zhou, Z., K. E. Pollok, K. K. Kim, Y. J. Kim & B. S. Kwon: Functional analysis of T-cell antigen 4-1BB in activated intestinal intra-epithelial T lymphocytes. *Immunol Lett* 41, 177-84. (1994)
239. Cannons, J. L., P. Lau, B. Ghumman, M. A. DeBenedette, H. Yagita, K. Okumura & T. H. Watts: 4-1BB ligand induces cell division, sustains survival, and enhances effector function of CD4 and CD8 T cells with similar efficacy. *J Immunol* 167, 1313-24. (2001)
240. Shuford, W. W., K. Klussman, D. D. Tritchler, D. T. Loo, J. Chalupny, A. W. Siadak, T. J. Brown, J. Emswiler, H. Raecho, C. P. Larsen, T. C. Pearson, J. A. Ledbetter, A. Aruffo & R. S. Mittler: 4-1BB costimulatory signals preferentially induce CD8+ T cell proliferation and lead to the amplification *in vivo* of cytotoxic T cell responses. *J Exp Med* 186, 47-55. (1997)
241. Gramaglia, I., D. Cooper, K. T. Miner, B. S. Kwon & M. Croft: Co-stimulation of antigen-specific CD4 T cells by 4-1BB ligand. *Eur J Immunol* 30, 392-402. (2000)
242. Mittler, R. S., T. S. Bailey, K. Klussman, M. D. Trailsmith & M. K. Hoffmann: Anti-4-1BB monoclonal antibodies abrogate T cell-dependent humoral immune responses *in vivo* through the induction of helper T cell anergy. *J Exp Med* 190, 1535-40. (1999)
243. Alderson, M. R.: Regulation of immune responses by the ligands for CD27, CD30, and 4-1BB. *Circ Shock* 44, 73-6. (1994)
244. Alderson, M. R., C. A. Smith, T. W. Tough, T. Davis-Smith, R. J. Armitage, B. Falk, E. Roux, E. Baker, G. R. Sutherland & W. S. Din: Molecular and biological characterization of human 4-1BB and its ligand. *Eur J Immunol* 24, 2219-27. (1994)
245. Blazar, B. R., B. S. Kwon, A. Panoskaltsis-Mortari, K. B. Kwak, J. J. Peschon & P. A. Taylor: Ligation of 4-1BB (CDw137) regulates graft-versus-host disease, graft-versus-leukemia, and graft rejection in allogeneic bone marrow transplant recipients. *J Immunol* 166, 3174-83. (2001)
246. Tan, J. T., J. K. Whitmire, K. Murali-Krishna, R. Ahmed, J. D. Altman, R. S. Mittler, A. Sette, T. C. Pearson & C. P. Larsen: 4-1BB costimulation is required for protective anti-viral immunity after peptide vaccination. *J Immunol* 164, 2320-5. (2000)
247. Tan, J. T., J. K. Whitmire, R. Ahmed, T. C. Pearson & C. P. Larsen: 4-1BB ligand, a member of the TNF family, is important for the generation of antiviral CD8 T cell responses. *J Immunol* 163, 4859-68. (1999)
248. DeBenedette, M. A., T. Wen, M. F. Bachmann, P. S. Ohashi, B. H. Barber, K. L. Stocking, J. J. Peschon & T. H.

Watts: Analysis of 4-1BB ligand (4-1BBL)-deficient mice and of mice lacking both 4-1BBL and CD28 reveals a role for 4-1BBL in skin allograft rejection and in the cytotoxic T cell response to influenza virus. *J Immunol* 163, 4833-41. (1999)
249. Melero, I., W. W. Shuford, S. A. Newby, A. Aruffo, J. A. Ledbetter, K. E. Hellstrom, R. S. Mittler & L. Chen: Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate established tumors. *Nat Med* 3, 682-5. (1997)
250. Guinn, B. A., M. A. DeBenedette, T. H. Watts & N. L. Berinstein: 4-1BBL cooperates with B7-1 and B7-2 in converting a B cell lymphoma cell line into a long-lasting antitumor vaccine. *J Immunol* 162, 5003-10. (1999)
251. Sica, G. & L. Chen: Biochemical and immunological characteristics of 4-1BB (CD137) receptor and ligand and potential applications in cancer therapy. *Arch Immunol Ther Exp* 47, 275-9 (1999)
252. Strome, S. E., B. Martin, D. Flies, K. Tamada, A. I. Chapoval, D. J. Sargent, S. Shu & L. Chen: Enhanced therapeutic potential of adoptive immunotherapy by *in vitro* CD28/4-1BB costimulation of tumor-reactive T cells against a poorly immunogenic, major histocompatibility complex class I-negative A9P melanoma. *J Immunother* 23, 430-7. (2000)
253. Kim, Y. J. & H. E. Broxmeyer: Therapeutic potential of 4-1bb (cd137) as a regulator for effector cd8+ t cells. *J Hematother Stem Cell Res* 10, 441-9. (2001)
254. Martinet, O., V. Ermekova, J. Q. Qiao, B. Sauter, J. Mandeli, L. Chen & S. H. Chen: Immunomodulatory gene therapy with interleukin 12 and 4-1BB ligand: long- term remission of liver metastases in a mouse model. *J Natl Cancer Inst* 92, 931-6. (2000)
255. Chen, S. H., K. B. Pham-Nguyen, O. Martinet, Y. Huang, W. Yang, S. N. Thung, L. Chen, R. Mittler & S. L. Woo: Rejection of disseminated metastases of colon carcinoma by synergism of IL-12 gene therapy and 4-1BB costimulation. *Mol Ther* 2, 39-46. (2000)
256. Wilcox, R. A., D. B. Flies, G. Zhu, A. J. Johnson, K. Tamada, A. I. Chapoval, S. E. Strome, L. R. Pease & L. Chen: Provision of antigen and CD137 signals to break immunological ignorance, promoting regression of poorly immunogenic tumors. *J Clin Invest (in press)*
257. Kim, Y. J., P. L. Mantel, C. H. June, S. H. Kim & B. S. Kwon: 4-1BB costimulation promotes human T cell adhesion to fibronectin. *Cell Immunol* 192, 13-23. (1999)
258. Kwon, B. S., C. A. Kozak, K. K. Kim & R. T. Pickard: Genomic organization and chromosomal localization of the T-cell antigen 4-1BB. *J Immunol* 152, 2256-62. (1994)
259. Vinay, D. S. & B. S. Kwon: Role of 4-1BB in immune responses. *Semin Immunol* 10, 481-9. (1998)
260. Chalupny, N. J., R. Peach, D. Hollenbaugh, J. A. Ledbetter, A. G. Farr & A. Aruffo: T-cell activation molecule 4-1BB binds to extracellular matrix proteins. *Proc Natl Acad Sci U S A* 89, 10360-4. (1992)
261. Goebeler, M., K. Kilian, R. Gillitzer, M. Kunz, T. Yoshimura, E. B. Brocker, U. R. Rapp & S. Ludwig: The MKK6/p38 stress kinase cascade is critical for tumor necrosis factor-alpha-induced expression of monocyte-chemoattractant protein-1 in endothelial cells. *Blood* 93, 857-65. (1999)
262. Flynn, S., K. M. Toellner, C. Raykundalia, M. Goodall & P. Lane: CD4 T cell cytokine differentiation: the B cell activation molecule, OX40 ligand, instructs CD4 T cells to express interleukin 4 and upregulates expression of the chemokine receptor, Blr-1. *J Exp Med* 188, 297-304. (1998)
263. Takahashi, C., R. S. Mittler & A. T. Vella: Cutting edge: 4-1BB is a bona fide CD8 T cell survival signal. *J Immunol* 162, 5037-40. (1999)
264. Nocentini, G., L. Giunchi, S. Ronchetti, L. T. Krausz, A. Bartoli, R. Moraca, G. Migliorati & C. Riccardi: A new member of the tumor necrosis factor/nerve growth factor receptor family inhibits T cell receptor-induced apoptosis. *Proc Natl Acad Sci U S A* 94, 6216-21. (1997)
265. Hurtado, J. C., Y. J. Kim & B. S. Kwon: Signals through 4-1BB are costimulatory to previously activated splenic T cells and inhibit activation-induced cell death. *J Immunol* 158, 2600-9. (1997)
266. Wilcox, R. A., A. I. Chapoval, K. S. Gorski, M. Otsuji, T. Shin, D. B. Flies, K. Tamada, R. S. Mittler, H. Tsuchiya, D. M. Pardoll & L. Chen: Expression of functional CD137 receptor by dendritic cells. *J Immunol (in press)*

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