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Commentary

Feasibility of Immunotherapy for Lymphangioleiomyomatosis

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The article by Klarquist et al in this issue of the *American Journal of Pathology* highlights commonalities between cells responsible for lymphangioleiomyomatosis (LAM) and melanoma tumors.[1] This work suggests that pulmonary lesions on LAM may therefore be susceptible to treatment by vaccines developed against melanoma.

Dismal Treatment Opportunities for Lymphangioleiomyomatosis

LAM strikes women during their reproductive years and leaves patients with a much reduced life expectancy at diagnosis.[2] Patients with LAM present with recurrent pneumothoraces, hemoptysis, pleural effusions, and dyspnea on exertion.[2] There is no truly effective treatment available beyond lung transplantation. LAM is definitively diagnosed by detecting expression of the glycoprotein recognized by the antibody HMB45.[3] Differential diagnosis is otherwise difficult, and patients with sporadic LAM typically remain undiagnosed for several years.[4]

Slow tumor growth and the paucity of patients with LAM has likely hindered the development of treatment strategies. Curiously, the development of LAM is restricted almost exclusively to women of childbearing age, suggesting that female hormones may be involved in disease development. Symptoms increase during pregnancy, and in postmenopausal women developing LAM, estrogen replacement therapy appears to contribute to disease development. Estrogens have been implicated in tumor metastasis in LAM.[5] Indeed, recent data support the notion that LAM tumors are capable of metastasis and may result from lymphatic deposition of tumor cells. As the main determinant of tumor development may lie in the balance of progesterone to estrogen, progesterone treatment has gained some popularity for LAM with limited success.[6]

A major breakthrough in LAM research has been the discovery that loss of heterozygosity in the tuberous sclerosis complex genes *TSC-1* and *TSC-2* underlies disease pathology.[7] Given the role of TSC-1 and TSC-2 in the mammalian target of rapamycin pathway, rapamycin treatment has been proposed for patients with LAM.[8] As rapamycin can reduce cell size but is not necessarily cytotoxic to tumors, this treatment modality apparently provides temporary relief of symptoms; yet further treatment is required to permanently eliminate lung tumors responsible for progressive dyspnea. However, in light of the current publication, the immunosuppressive effects of rapamycin should be considered as well. Although rapamycin may inhibit tumor growth, after treatment it may be more difficult to elicit immune responses to the remaining viable cells.[9]

Expression of Melanoma Differentiation Antigens

The cells that originate LAM remain an enigma, and both endothelial cells and smooth muscle cells are candidate cell types to be transformed in LAM.[3] Tumor recurrence in transplanted lung tissue is supportive of clonality and metastasis of the lesions.[10] Patients with LAM frequently present with renal lesions as well, and expression of melanoma-associated antigens has also been reported in renal lesions. The fact that gp100 is expressed by the tumor cells further complicates this issue and suggests developmental similarities to melanocytes, the pigment-generating cells of the skin. However, such expression may render the tumor susceptible to melanoma-reactive T cells, and herein lies the significance of the paper in this issue of the *AJP*.

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Melanoma Vaccines

Current immunotherapeutic concepts have been developed primarily based on observations in melanoma. This deadly skin tumor is uniquely immunogenic, and the abundance of lymphocytes found in melanoma tumors has provided an opportunity to isolate and propagate tumor-specific T cells for adoptive therapy.[11] In addition, *in vitro* studies using tumor-infiltrating lymphocytes and cultured melanoma cells have provided opportunities to characterize target molecules and target epitopes preferentially recognized by T cells. Melanoma immunotherapy can be further optimized to enhance peptide binding to Major Histocompatability Complex to provide a stronger stimulus for T cell activation and by identification and cloning of T cell receptor subunits that may be introduced into patient effector cells to redirect the immune response toward the tumor.[12,13] The article by Klarquist et al explores the expression of gp100 and other melanoma-associated antigens in LAM with the ultimate objective of exploring the opportunity of using melanoma immunotherapy for the treatment of LAM.

TRP-1 as a Diagnostic Marker

Besides gp100, the MART-1 protein as well as tyrosinase and TRP-2 have proven to be highly immunogenic molecules that can evoke effective antitumor responses in mice and humans. As shown in this issue of the *AJP*, expression of gp100 and MART-1 in LAM cells is accompanied by the expression of additional melanoma-associated antigens TRP-1 and TRP-2. Quantifying the expression in lung lesions, antibodies to TRP-1 appeared to detect the greatest proportion of lesional cells, and the authors argue that such antibodies may provide a more sensitive marker for LAM diagnosis. It should be noted, however, that currently available antibodies to TRP-1 do not appear to work well on paraffin-embedded tissue. Another noteworthy finding includes the observation that individual melanoma-associated markers are not necessarily expressed by the same subset of LAM cells. This indicates that a combination of melanoma-associated antigens should be included in future vaccines against LAM to target the greatest possible proportion of tumor cells. Melanoma vaccines have targeted MART-1, gp100, tyrosinase, TRP-2, and TRP-1, strikingly all of which are part of the melanin synthesis machinery in pigment cells.[14]

Immune Infiltration of LAM Tissue

The expression of highly immunogenic molecules in LAM raises the question of whether tumor growth is, like melanoma, accompanied by lymphocyte infiltration. The authors addressed this issue by characterizing the immune infiltrates in LAM versus normal lung, also including a comparison to melanoma. The authors observed a striking infiltration by macrophages, which is in concordance with the macrophage infiltration of tuberous sclerosis lesions described previously.[15] However, there were few, if any, signs of ongoing adaptive immunity in LAM lungs. Moreover, macrophage infiltration is generally associated with a poor prognosis in different tumor types.[16]

T cells are generally abundant in the lung as part of an active immune surveillance, but no increases were observed in LAM lungs. Dendritic cells were equally absent, suggesting that the immune system is not alerted to the occult expression of melanoma antigens in LAM lungs. Most importantly, cultured LAM cells were susceptible to T cell-mediated killing using melanoma-derived T cells. Taken together, the data demonstrate that expression of melanoma-associated antigens renders LAM cells susceptible to T cell-mediated immune responses, yet such responses are not commonly elicited *in vivo*.

Pulmonary Immune Responses

The data shown in the article by Klarquist et al[1] suggest that immunotherapy may be a new potential avenue to treat LAM. There are, however, specific issues to be dealt with before vaccination of patients with LAM can be translated to the clinic. Specifically, thoracic oncology is a particularly challenging field to be approached by vaccines. In small-cell lung cancer, antigens such as GD3 have been targeted with limited success.[17] The success of targeting these and other antigens by conventional tumor vaccines appears to be limited by the pulmonary environment.[18]

Like the skin, the lungs are exposed to external pathogens and are thus equipped to efficiently deal with infectious agents and other pathogens without loss of vital lung function. Mucociliary clearance offers a first line of defense against pathogens without alerting the immune system, at which point innate immune responses may be activated.[19] Pathogens that evoke tissue damage and cause inflammation will encounter an abundance of immune cells including alveolar macrophages, dendritic cells, and T cells, yet how their efficacy compares with the same cell types resident in other tissues largely remains to be described. For example, CD4/CD8 double positive cells appear to be relatively abundant in pulmonary tissue, the significance of which has not been studied; whereas, curiously, CD4/CD8 double negative T cell infiltration appears to be relevant to the maintenance of lung transplant physiology.[20]

The data presented in the article suggest that pulmonary immunosurveillance does not spontaneously initiate in LAM despite the occult expression of highly immunogenic molecules. It may be expected that renal lesions will clear more frequently given the relative immunogenicity of renal cell carcinomas.[21] In fact, it is possible that the absence of renal angiomyolipomas in some LAM patients is reflective of effective responses to such lesions. It seems possible that targeting LAM by antitumor vaccines will require the design of tissue-specific vaccines.

Conclusion

In all, the data provided by Klarquist et al[1] are supportive of the concept that LAM may be targeted by vaccines

directed against melanoma-associated differentiation antigens. Multiple molecules described as highly immunogenic in melanoma tumors were found in LAM, and the TRP-1 molecule was highly expressed, even in comparison with melanoma. At this time, the basis for occult expression of melanoma-associated antigens by transformed cells in LAM lung is not understood. Special adaptations may be required to direct immune responses to the lung. Further research is certainly necessary, but this new direction in LAM research is very promising.

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