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Mechanism of Immune Response During Immunotherapy

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Tumor immunology embraces an extensive array of biological phenomena that include interactions between neoplastic cells and the innate and adaptive immune response. Among immune cells, T cells have taken the center stage because they can be easily demonstrated to specifically recognize autologous cancer cells. However, their role is limited and other components of the immune response are likely necessary for the completion of cancer rejection.

Metastatic melanoma and renal cell carcinoma (RCC) are malignancies strongly predisposed to regress in response to the systemic administration of high-dose interleukin (IL)-2. Several clinical Studies in extensive cohorts of patients have shown that this treatment can induce complete or partial clinical regressions of metastatic disease in 15 to 20% of patients who receive this treatment.[1-6] Although IL-2 has direct pluri-potent effects on cells with immune and inflammatory function, it remains unexplained which cell subset is implicated in mediating tumor regression.

In a quest to characterize the mechanism of action of IL-2 during the course of immunotherapy, we have investigated the early changes in transcriptional profiles of circulating mononuclear cells and microenvironment of melanoma metastases following high dose IL-2 administration (720,000 IU/kg) by serial sampling of blood cells and tumors in the form of fine needle aspirate (FNA).[7] Furthermore, studies are currently ongoing to characterize the proteomic profiling of RCC patients undergoing the same treatment using protein arrays (manuscript in preparation). The predominant activation of genes related to inflammation and activation of mononuclear phagocytes lead us to further characterize this cell subset in the context of stimulation with a panel of soluble factors potentially present in the circulation and tumor microenvironment.

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Gene Profiling of Systemic and Local IL-2 Responses

We recently reported on the gene profiling of mononuclear cells and microenvironment of melanoma metastases following systemic IL-2 administration.[8] By comparing early changes in transcriptional profiles of circulating mononuclear cells with those occurring within the microenvironment of melanoma metastases following systemic IL-2 administration we observed that the immediate effects of IL-2 administration on the tumor microenvironment is transcriptional activation of genes predominantly associated with monocytic cell function while minimal effects were noted on migration, activation and proliferation of T cells. However, production of chemokines and markers of adhesion and migration within few hours of IL-2 administration may be responsible for a secondary recruitment of immune cells to the tumor site at a later time point. This study suggested that IL-2 administration induces inflammation at tumor site with the three predominant secondary effects of activation of antigen presenting monocytes, massive production of chemoattractants that may recruit other immune cell to the tumor (MIG, PARC) and activation of lytic mechanisms (calcgranulin, grancalcin) ascribable to monocytes and NK cells (NKG5, NK4).

Furthermore we have recently reported on the complexity and individual variability of the biological response (secretion of soluble proteins) in PBMC from 14 individuals (13 melanoma patients and 1 normal donor) stimulated *ex vivo* with IL-2 (6,000 IU/ml of IL-2).[9]

To investigate *in vivo* the effect of IL-2 administration on proteins released in the circulation, we

conducted a small pilot study on the profile of 68 cytokines, chemokines and soluble factors present in the serum of RCC patients undergoing high dose IL-2 administration (manuscript in preparation). Serum was collected pre, 3 hrs post 1st dose and 3 hrs post 4th dose of IL-2 and screened by multiplexed protein array platforms (Searchlight TM, Boston, MA). We observed an outburst of a multitude of proteins increasing in the circulation in response to IL-2. Fifty six percent of the soluble factors tested were significantly increased in the circulation post one dose in 10 patients, 61% in a total of 15 patients. Seventy-six percent of the factors were increased post 4 doses and 59% were significantly different between 1 and 4 doses of IL-2.

Global Gene Profiling of LPS Activated Mononuclear Phagocytes Stimulated with Soluble Factors Potentially Present in the Circulation and Tumor Microenvironment

The gene profiling studies we conducted on patients undergoing IL-2 administration revealed that mononuclear phagocytes (MP) play a critical role in the tumor microenvironment of melanoma patients.[8] These studies suggested that MP stand at the crossroads between the induction of acute inflammation to recruit and activate immune effector cells and the down- modulation of the inflammatory process to contain collateral damage in the occurrence of a pathogenic occurrence as well as in the tumor microenvironment. This decision is extensively modulated by the cytokine microenvironment that includes a broad array of cytokines whose direct effect on MP remains largely unexplored. Therefore, we tested whether polarized responses of MP to pathogens are related to the influence of selected cytokines or represent a mandatory molecular switch through which most cytokines operate (Nagorsen et al, submitted 2002). Circulating CD14+ MP were exposed to i) LPS followed by ii) exposure to an array of cytokines, chemokines and soluble factors involved in the immune response. Gene expression was studied by global transcript analysis. Two main classes of cytokines were identified that induced a classical or an alternative pathway of MP activation. Expression of genes affected by NF-κB activation was most predictive of the two main classes suggesting that this pathway is a fundamental target of cytokine regulation. Since LPS itself induces a classical type of activation, the most dramatic modulation was observed toward the alternative pathway suggesting that a broad array of cytokines may counteract the pro-inflammatory effects of bacterial components.

Conclusion

Complex biological processes are occurring in a tumor microenvironment subjected to immune pressure, therapy and genetic instability. Tumor heterogeneity can now be studied in its globality using state of the art genomics and proteomics tools.

We have attempted to dissect the mechanism of immune response to high dose IL-2 administration by gene profiling and proteomic studies using peripheral blood and serial fine needle aspirate of melanoma metastasis. We are only at the very beginning of an exciting quest to unravel the enigma of IL-2 efficacy and toxicity. Our gene profiling and proteomic studies have revealed that an outburst of multiple inflammatory factors are involved in IL-2 activity and effects. Furthermore, mononuclear phagocytes appear to be a critical player in IL-2 induced immune response to tumor.

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