(2) T-CELL GROWTH FACTORS

Interleukin-15 (IL-15)

Presenter: Jay Berzofsky, M.D., Ph.D.

Interleukin-15 (IL-15) is a four-helix-bundle cytokine similar to IL-2. It is made by DCs, macrophages, and stromal cells, but not by T cells. It acts on CD8+ T cells, CD4+ T cells, natural killer cells, and mast cells. It binds to a unique IL-15 receptor alpha chain; whether this is presented in *cis* or *trans* configuration affects how IL-15 functions.

IL-15 inhibits antigen-induced cell death (AICD) of T cells, in contrast to IL-2, which promotes AICD. In vaccines, it promotes induction of longer-lived and higher-avidity CD8+ T cells that kill tumor cells very effectively. It is not just a matter of maintaining T-cell memory. IL-15 selects for a different population of cells. Greenberg's group showed that IL-15 can reverse T-cell anergy. Dr. Palucka demonstrated that IL-15 promotes *in vitro* differentiation of monocyte-derived DCs that are potent inducers of CD8+ T cells. Unpublished data from Dr. Berzofsky's lab indicate that IL-15 can overcome lack of CD4 help in CTL induction. Data from Dr. Khleif and Drs. Pavlakis and Felber show that IL-15–expressing plasmids can induce tumor regression in mice via intratumoral injection or hydrodynamic delivery. No clinical data are available.

As a vaccine adjuvant, it might be used to induce longer-lived, higher avidity, more efficacious CD8+ T cells. As a single agent, it could potentially be used to overcome T-cell anergy and could be used in place of IL-2 as a T-cell growth factor to sustain adoptively transferred T cells. It would be useful also for *in vitro* differentiation of dendritic cells to use as cellular vaccines.

The risk-benefit profile would have to be taken into account if it were to be used as a systemic agent due to its side-effect profile (e.g., cytokine storm).

IL-15 could have an important role for cancer vaccines and adoptive immunotherapy, as well as for direct therapy *in vivo* and for DC differentiation *in vitro* for DC vaccine therapy. At least 10 investigators are working to obtain GMP-grade IL-15 for clinical use.

Discussion

Dr. Mackall agreed that IL-15 could have an important role in many areas of interest, including adoptive T-cell therapy. Paul Sondel, M.D., Ph.D., said that IL-15 might also be a potent activator of NK cells without up-regulating Tregs. The agent has been the subject of preclinical investigations for quite some time.

Investigators have encountered a number of barriers when attempting to initiate trials. Several participants discussed the lack of IL-15 availability for conducting clinical trials. According to Dr. Calzone, Amgen has released several such molecules for preclinical studies, but clinical trials are costly and entail a great deal of work. Dr. Weber emphasized the importance of a first-in-human trial to garner some toxicity and safety data as a critical first step before commencing other trials.

Jeffrey Schlom, Ph.D., brought up the topic of vector-driven agents and emphasized the importance of keeping them under consideration by this group. Pharmaceutical firms might possibly be more willing to go down those paths.

Recently, several groups have shown that IL-15 is more potent and stable when given in combination with IL-15 receptor alpha. IL-15 is a very interesting cytokine, but more than one form exists.

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Interleukin-7 (IL-7)

Presenter: Crystal Mackall, M.D.

Interleukin-7 is required for T-cell development in humans and for naive T-cell survival in the periphery. IL-7 signaling or T-cell activation results in receptor down-regulation—an effect opposite to that of IL-2 and IL-15. IL-7 signaling on mature T cells leads to homeostatic expansion of naïve cells during lymphopenia.

The IL-7 receptor is present throughout T-cell development but not on effector or senescent cells. Receptor expression marks cells destined to become memory T cells during the evolution of the immune response.

Preclinical studies have established IL-7's usefulness as a vaccine adjuvant. The agent enhances CD4+ and CD8+ effector and CD8 memory populations but does not have much of an effect on myeloid or B cells. The most dramatic effects occur on the subdominant responses.

Proof-of-principle has been established in phase I trials conducted with cancer patients. Dramatic increases in total body CD4+ and CD8+ T cells, as well as modest increases in natural killer cells have been observed. No selective increase in Tregs occurs. No significant toxicities have been reported.

This agent could be potentially useful as a means to restore T cells after bone marrow transplantation. Improving immune reconstitution in this setting may diminish leukemia relapse. Also, combining IL-7 with Treg depletion has shown therapeutic benefit in the setting of

adoptive immunotherapy for B16 melanoma. However, whether IL-7 will be in adoptive cell therapy remains untested because many cells will not express the receptor. Alternatively, patients who experienced a beneficial effect from adoptive cell therapy in studies conducted by Steve Rosenberg have a modest increase in IL7 receptor expression compared to those in whom adoptive cell therapy is not effective. Therefore it remains possible that selective expansion of IL7R-expressing T cells could improve the effectiveness of adoptive cell therapy and studies are needed to assess this.

IL-7 administration preferentially expands the pool of naïve T cells. Given that older people are the ones more likely to get cancer, it is remarkable how many of them develop naïve cells when given IL-7.

In response to a question from a participant, Dr. Mackall said that she was not sure what percentage of T cells generated after a peripheral stem cell transplant express low levels of IL-7 receptor. Naïve cells from the thymus would expand in this setting. IL-7 promotes low-avidity T-cell responses. Other questions dealt with the risk of autoimmune disease occurring in cancer patients treated with IL-7. Neutralizing antibodies merit close attention because IL-7 is such a good adjuvant.

Discussion

Dr. Ho said that IL-7 really should be in its own category because of its profound effect on naïve cells. An ongoing trial is seeking answers to this research question in the context of adoptive immunotherapy, which entails immunodepletion beforehand.

Dr. Whitehead said that she thinks of IL-7 as a survival cytokine. It might be a very good addition to antitumor vaccines. Dr. Berzofsky said that if the induced effector cells do not have IL-7 receptor, it is not clear that the agent would have a survival effect on those cells.

A discussion ensued about using IL-7 and IL-15 in combination because, at least in theory, IL-15 would be effective after the expansion effect. At least one study found, however, that giving the two cytokines together showed no additive effect.

Some investigators have access to IL-7, and NCI has a repository available. The Institute was able to provide the agent for toxicity studies and gave manufacturing guidance to a biotech company, Cytheris, which is now producing it and sponsoring a couple of trials in areas of HIV treatment and bone marrow transplantation. Production problems appear to have been resolved.

An Italian group is studying the agent's use in children with IL-7 receptor alpha deficiency.

Mouse knockouts are more susceptible to carcinogenesis because they resemble mice with severe combined immunodeficiency (SCID).

Dr. Amy Rosenberg inquired whether solid tumor cells express IL-7 receptor. Apparently, it is expressed in early B-cell lymphoid cancers and lung cancer.

By voice acclamation, the participants determined the priority ranking of the T cell growth factors to be IL-15, IL-7. According to the participants, both agents are very interesting, and they hoped that both could be made available.

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Interleukin-21 (IL-21)
Presenter: William Ho, M.D., Ph.D.

Dr. Ho emphasized that he is not speaking for Genentech; rather, he is offering his personal point of view. IL-21 is not a Genentech product.

A member of the common gamma-chain family of cytokines, IL-21 induces and preserves CD28+ T cells. It also has been found to improve the degree of expansion and affinity of antigen-specific CTL clones generated *in vitro*. It is produced primarily by CD4+ T cells. The receptor is expressed by T cells, B cells, natural killer cells, DC/myeloid cells, and non-immune cells. Recent work has demonstrated IL-21's involvement in inducing differentiation of pro-inflammatory murine CD4+ T_H17 cells. It can also induce apoptosis in natural killer cells. Perhaps counterintuitive to its immunostimulatory function with CD8+ T cells, it can inhibit maturation, activation, and differentiation of DCs, producing an immunosuppressive phenotype. It also causes apoptosis in naïve or incompletely activated B cells.

In vitro studies have shown that IL-21 can promote apoptosis in B-CLL cells but has also been shown to induce proliferation, and it inhibits apoptosis in some acute T-cell leukemia and multiple myeloma cell lines.

Investigations of *in vivo* models have indicated that IL-21 has activity in multiple tumor types, causing tumor rejection, preventing metastases, and enhancing immune memory.

Several phase I trials of IL-21 in metastatic melanoma and renal cell carcinoma have been conducted. Objective response rates of < 10% were seen (one partial remission in renal cell carcinoma, one complete remission in melanoma; the majority experienced stable disease). A phase IIa trial in melanoma is under way. Other phase I or II studies, either planned or ongoing, include IL-21 in combination with rituximab (anti-CD20), sorafenib, or cetuximab (anti-epidermal growth factor receptor).

IL-21 might be used in the clinic as a systemic immunomodulator in monotherapy or in combination with antibody-dependent cell-mediated cytotoxic (ADCC) agents. It is also of interest as a cancer vaccine adjuvant and for cultivating CD8+ cells or clones for adoptive T-cell transfer.

Discussion

Kim Margolin, M.D., observed that IL-21 went quickly into human trials from preclinical work. She speculated that demand might not be as great for this agent as many others because of the lack of preclinical data demonstrating its potential. The company that holds the intellectual property is doing a good job of collecting biomarkers. IL-21 is in active clinical development, in contrast to IL-15.

Dr. Urba said that IL-21 may have an effect on memory cells and differential effects—features that make study of this cytokine important. Expansion of memory cells is the important factor.

Dr. Berzofsky reported observing some synergy with IL-15 in a collaborative study with Warren Leonard's lab (See Zeng et al. below).

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Discussion of T-cell Growth Factor Prioritization

By voice acclamation, the priority ranking of all the T-cell growth factors discussed was determined to be:

1. IL15
2. IL7
3. IL21