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ABSTRACT

The Transforming Growth Factor beta (TGF- β) superfamily includes three isoforms designated TGF- β 1, β 2 and β 3. All three isoforms are secreted as latent complex where the TGF- β cytokine is non-covalently associated with an isoform specific latency-associated peptide (LAP). Mature cytokine binds cell surface receptors only after release from its LAP making extracellular activation a critical regulatory point for TGF- β bioavailability. Proposed activation mechanisms include proteolysis and conformational changes. Previous work from our laboratory showed that latent TGF- β 1 (LTGF- β 1) is efficiently activated upon exposure to reactive oxygen species (ROS).

ROS activation is restricted to the LTGF- β 1 isoform. Because of the amino acid sequence differences between the three LAPs, we postulate that the specificity of this activation mechanism lies within the LAP. Furthermore, we hypothesize that the presence of a metal in the latent complex could provide a redox active center for this process.

Redox mediated activation provides a novel mechanism for TGF- β participation in tissues undergoing oxidative stress. Moreover, this would allow TGF- β 1 to act both as a sensor of oxidative stress within tissues as well as a transducer of that signal by binding to its cellular receptors.

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Introduction

The Transforming Growth Factor beta (TGF- β) superfamily includes three isoforms designated TGF- β 1, β 2 and β 3. All three isoforms are secreted as latent complex where the TGF- β cytokine is non-covalently associated with an isoform specific latency-associated peptide (LAP). Mature cytokine binds cell surface receptors only after release from its LAP making extracellular activation a critical regulatory point for TGF- β bioavailability. Proposed activation mechanisms include proteolysis and conformational changes. Previous work from our laboratory showed that latent TGF- β 1 (LTGF- β 1) is efficiently activated upon exposure to reactive oxygen species (ROS).

ROS activation is restricted to the LTGF- β 1 isoform. Because of the amino acid sequence differences between the three LAPs, we postulate that the specificity of this activation mechanism lies within the LAP. Furthermore, we hypothesize that the presence of a metal in the latent complex could provide a redox active center for this process.

Redox mediated activation provides a novel mechanism for TGF- β participation in tissues undergoing oxidative stress. Moreover, this would allow TGF- β 1 to act both as a sensor of oxidative stress within tissues as well as a transducer of that signal by binding to its cellular receptors.

Specific Aims:

1. To characterize the interaction between reactive oxygen and latent TGF- β
2. To identify and localize the redox-metal center within the latent TGF- β
3. To determine the three-dimensional structure of latent TGF- β

Progress:

Aim 1. To characterize the interaction between reactive oxygen and latent TGF- β

We have collected data to show that activation by ROS is restricted to the LTGF- β 1 isoform. Due to the high degree of similarity between the amino acid sequences of the three cytokines (TGF- β 1, β 2 and β 3), it is not likely that the redox center resides within the cytokine region. However, amino acid sequences of the three LAPs are not highly conserved suggesting that the redox center specific to LTGF- β 1 may reside within LAP- β 1. Methionine residues are particularly susceptible to oxidation, and there are three non-conserved methionine residues (at positions 112, 132, and 253) within LAP- β 1. As mentioned in last years report, in collaboration with Dr. Dan Rifkin at NYU, we have developed a series of mutants where these methionine residues in LTGF- β 1 have been mutated to alanine. These mutants have been transiently expressed in mammalian cells and conditioned medium has been collected and characterized for ROS activation using the PAI-luciferase assay to measure the amount of active TGF- β . Results from these experiments suggest that methionine 253 may be critical in the ROS activation mechanism. In addition to this methionine residue, other amino acid residues may also be involved in the redox center. In order to identify and characterize these, we have

begun collaborating with Dr. John Shively and Dr. Terry Lee at the City of Hope, Beckman Research Institute. LTGF- β 1 protein will be subjected to activation by ROS. Using mass spectrometry peptide mapping, amino acids modified by the ROS activation will be identified. We are preparing for the possibility that large amounts of protein may be required. Currently, we have LTGF- β 1 stably expressed in CHO cells. We also have the capacity to scale up the cultures and protein production by culturing the cells in roller bottles.

Aim 2. To identify and localize the redox-metal center within the latent TGF- β

After the identification of amino acids involved in the redox center, we will further explore this center for the presence of metal. The presence of a metal may be detected by the use of inductively coupled plasma mass spectroscopy (ICP-MS). This possibility will be explored in our collaboration with Dr. John Shively and Dr. Terry Lee.

Aim 3. To determine the three-dimensional structure of latent TGF- β

In collaboration with Dr. Peter Walian here at LBNL, the crystal structure of LTGF- β 1 is being determined. Currently in the laboratory, we have a large amount of expressed and purified LTGF- β 2. We have been using this material to test crystallization conditions. LTGF- β s are glycosylated proteins and the sugar moieties can interfere with crystallization. Thus, LTGF- β 2 has been methodically deglycosylated in a manner that retains latency and native conformation. This deglycosylation method is currently being scaled-up in order to obtain the amounts of deglycosylated protein required for crystal formation. Furthermore, small-scale crystallization trials have begun with the deglycosylated LTGF- β 2 to determine the buffer conditions required for crystal formation. Because the ultimate goal is to crystallize LTGF- β 1, scale-up of the production of LTGF- β 1 protein is underway. We have stably expressed LTGF- β 1 in CHO cells. CHO cells were chosen because much of the literature on LTGF- β 1 uses these cells for production of the protein. These cells have been expanded into roller bottles and once confluent, they will be maintained in medium specifically designed for purification of expressed proteins secreted into the conditioned medium. This medium, purchased from Gibco/Invitrogen, is a low protein-containing medium that does not contain LTGF- β 1 as determined by the PAI-luciferase assay. Once large amount of conditioned medium have been collected, the LTGF- β 1 will be purified using previously published methods. Crystals will be made from the purified LTGF- β 1 using the conditions defined in the LTGF- β 2 crystallization experiments. Structural information of LTGF- β 1 and LTGF- β 2 is important in order to completely characterize the redox-center in LTGF- β 1.

Key Research Accomplishments

1. Stable expression of LTGF- β 1 for use in biochemical and structural studies
2. Deglycosylation of LTGF- β 2 to prepare for crystallization and progress in determining conditions required for crystallization of LTGF- β 2 and LTGF- β 1

Reportable Outcomes

None to report this year

Additional Comments

In this past year Dr. Michael Jobling has left this Post-doctoral position to pursue an industrial career in his home country of Australia. However, we are actively recruiting a Post-doctoral Fellow to continue this important research. Since Michael's departure, Dr. Joni Mott, a Scientist in the laboratory, has been overseeing the maintenance of the experiments so that the work accomplished has not been wasted. This will also provide a smooth and seamless transition of the work to the newly appointed Fellow.