IL-7 and IL-7R expression in PB mononuclear cells, PB monocytes or differentiated macrophages of RA patients with active vs. inactive disease treated with DMARD and/or CIMZIA.
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1. Background and Purpose

Monocyte migration plays a key role in the pathogenesis of Rheumatoid arthritis (RA) since the number of monocyte derived macrophages is higher in RA synovial tissue compared to normal joints and is well correlated with radiological damage, joint pain and inflammation. However, the signaling pathway(s) responsible for enhanced recruitment of monocytes into the RA synovial joint space is unknown. To address this important issue, we performed microarray studies to identify differentially regulated genes in RA synovial fluid macrophages and identified IL-7 Receptor (IL-7R) as one of the most highly upregulated genes in RA synovial fluid macrophages. Our preliminary data shows that IL-7R expression is elevated 45 fold in RA synovial fluid and 10 fold in RA peripheral blood (PB) *in vitro* differentiated macrophages compared to normal PB macrophages by real-time RT-PCR and/or FACS analysis. Consistently, in RA synovial tissue, IL-7R is also elevated on macrophages in the lining and sublining compared to normal synovial tissue. We found that expression of IL-7R in RA PB monocytes and macrophages is modulated by TNF-α Interestingly, concentrations of IL-7R have a strong correlation with disease activity score as determined by 28 joint count (DAS28) and TNF-α levels in RA monocytes. Moreover, our preliminary data documents that IL-7 is secreted from TNF-α treated RA macrophages and can attract IL-7R+ monocytes from the circulation to the RA joint at concentrations of IL-7 detected in RA synovial fluid.

Based on our results we hypothesize that RA patients with a TNF-α driven active disease have elevated expression of IL-7/IL-7R and thereby response to anti-TNF-α therapy reduces IL-7-mediated monocyte migration and the number of IL-7R+macrophages in the RA joint. To test our hypotheses, in aim 1 we will examine whether expression of IL-7 or IL-7R in RA PB cells correlates with RA disease severity and response to anti-TNF-α treatment (Fig. 1). To date the contribution of IL-7 and IL-7R to RA pathogenesis and its role as a TNF-α responsive gene has not been described.

IL-7R is the top gene identified in synovial fluid macrophages and its expression in monocytes (R^2=0.56 and p=8.4x10^{-5}) and macrophages (R^2=0.56 and p=2.4x10^{-6}) strongly correlates with RA disease activity suggesting that IL-7R may be a predictor for disease severity. Hence in this proposal, novel translational studies will be performed in order to determine whether IL-7R and IL-7 are RA signature genes for disease activity and if these genes are markedly reduced in anti-TNF-α therapy responders compared to nonresponders.

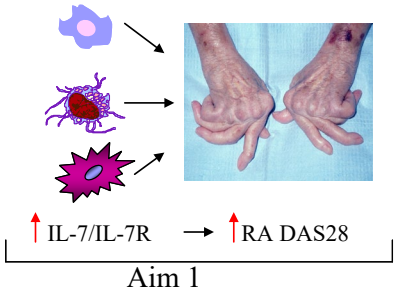


Figure 1. Specific aim is proposed to elucidate the significance of IL-7/IL-7R expression in peripheral blood cells to RA disease activity and response to anti-TNF-α treatment.

To accomplish the proposed project we will:

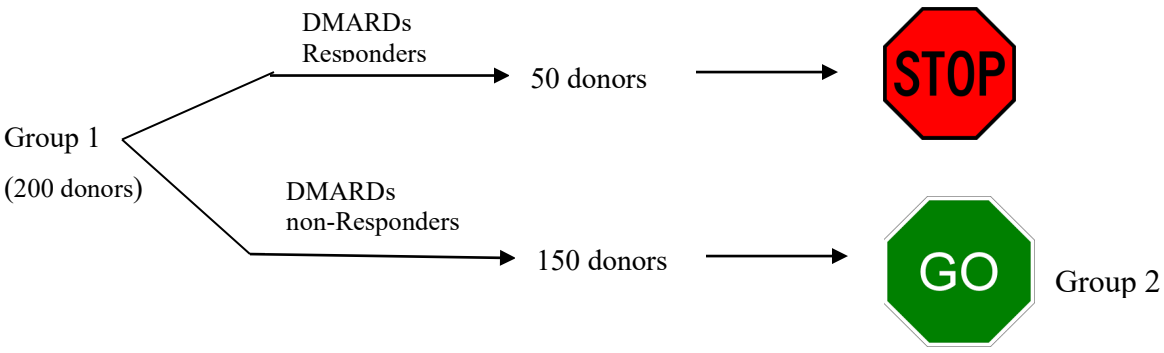
Aim 1. Determine whether the levels of IL-7 and IL-7R are elevated in PB mononuclear cells, PB monocytes or differentiated PB macrophages of RA patients with active [disease activity score (DAS)28>2.6] and inactive disease (DAS28<2.6) treated with Disease modifying anti rheumatic drugs (DMARDs) alone and/or DMARDs plus Cimzia® (certolizumab Pegol).

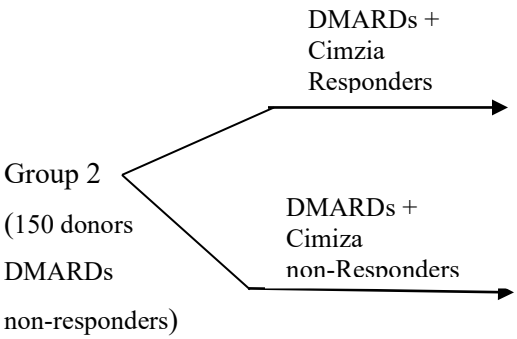
2. Protocol Methodology

Detection of IL-7 and IL-7R expression levels in RA patients with active and inactive disease: RA patients will be referred and informed written consent will be obtained by Latriese Sardine. Blood samples will be collected from patients that have been diagnosed with RA based on ACR classification criteria. The study will include 200 donors. The total number of subjects are divided into two groups to yield a power of 95% at a type I error 5% level [determined based on the preliminary data]. In the first group, 200 donors will be treated with methotrexate, plaquenil and/or prednisone (DMARDs) that either achieve remission (DAS28<2.6) or do not achieve remission (DAS28>2.6). 50 donor will be utilized as they respond to DMARDs and achieve remission (DAS28<2.6) and 150 donors that do not respond to DMARDs will be transferred to second group. In the second group, 150 donors will be treated with methotrexate, plaquenil and/or prednisone and Cimzia® (provided to us by UCB).

In the first group of patients, blood samples will be obtained from RA patients treated with Disease modifying anti rheumatic drugs (DMARDs) such as methotrexate, plaquenil and/or prednisone that achieve remission (DAS28<2.6). The patients that achieve remission (DAS28<2.6), blood will only be taken once at the patients routine visit.

The second group will consist of RA patients that did not respond to "DMARDs". These patients will further receive (DMARDs) such as methotrexate, plaquenil and/or prednisone as well as Cimzia® (provided to us by UCB) free of charge. Cimzia® is a FDA approved drug and is a standard of care. Blood samples will be obtained from the patients treated with DMARDs including methotrexate, plaquenil, and/or prednisone and Cimzia® (provided to us by UCB) that have inactive remission (DAS28<2.6). In this group, blood samples will be collected onset of the study as well as 3 and 6 months after treatment with Cimzia at patient's visit through our collaboration with the aforementioned rheumatologists. Patients receiving intra-articular steroid injections will be excluded from the study.





PB mononuclear cells will be isolated from RA whole blood and drawn into EDTA or CPT tubes and isolated by Histopaque gradient centrifugation. Monocytes will be isolated from RA PB mononuclear cells by negative selection (as shown in the preliminary data) and half of the monocytes will be differentiated to macrophages for 7 days. The expression levels for IL-7 and IL-7R will be determined by real-time RT-PCR and FACS analysis.

In our statistical analysis, we will first perform a stratified analysis to evaluate the differential expression levels in RA patients with active and inactive disease, controlling for the type of treatment. Data analysis will be performed in collaboration with an UIC Center for Clinical and Translational Science statistician. Specifically, we will perform the comparison of IL-7 and IL-7R expression among RA patients with active (DAS28>2.6) vs. inactive disease (DAS28<2.6) for DMARDs group (group 1). We will then perform a similar comparison for the DMARDs and Cimzia® therapy group (group 2). The stratified analysis can adjust for the potential confounding effect of treatment received and allows for the detection of the potential differential relationships between expression levels of IL-7 or IL-7R and disease status. We will also perform a pooled regression analysis in which the expression logarithm of IL-7 or IL-7R from patients is regressed on the treatment group indicator [DMARDs (group 1) versus on DMARDs plus Cimzia® therapy (group 2)] and disease status (active or inactive disease) which would demonstrate the interaction between treatment groups and disease activity. Such an analysis pools subjects from two treatment groups together and can therefore increase the sample size, and hence potentially the power of detecting the relationships between biomarkers and disease status. The RA samples will be collected over a 2 year period and the data will be analyzed in the last year of the proposal.

Measure outcomes: For any of the genes that demonstrate at least 2.5 fold increase (89% sensitivity calculated based on our preliminary data) in active disease compared to remission, correlation coefficient will be calculated for that gene expression level, rheumatoid factor, Cyclic Citrullinated Peptide Antibody (anti-CCP), C-Reactive Protein (CRP), DAS28 and disease duration.

3. Eligibility Criteria

Subject inclusion criteria for RA blood:
1. Must meet 1987 Revised Criteria for the Classification of Rheumatoid Arthritis defined as the diagnosis of the referring physician.
2.. Persistent knee swelling (>ARA grade 2) for 2 weeks, and no recent intra-articular corticosteroid injection.
3. Age 18 years and older.

4. Exclusion Criteria:

1. Patients having received intra-articular corticosteroid joint injection within the last 2-4 weeks.
2. Patients with active systemic or joint infections.
3. Women who are pregnant (pregnancy status will be self-reported)
4. Patients under 18 years of age
5. Non-English speakers

5. Vulnerable Population Inclusion:

1. No vulnerable population will be included in this study.
2. RA patients treated with Cimiza have to practice abstinent.

6. Probable Duration of Protocol

The samples will be stored for 10 years or until total sample depletion.

7. Location Where Research is to be Conducted

Recruitment will be conducted at the Outpatient Care Center, 1801 West Taylor Street, Suite 3A, Chicago IL, 60612. Research work done on samples will be conducted in the laboratory space in 835 S. Wolcott St, Room E807 MSB, University of Illinois at Chicago, Chicago IL, 60612.

8. Special Precautions to be Taken by Researchers

The potential risks are limited to that associated with routine blood draw, and the collection and storage of personal health information.

A possible risk of the research is that your participation in the research or information about you and your health might become known to individuals outside the research.

Latriese Sardine will obtain consent from patients with an informed consent document and authorization form. Copies of these forms will be added to each patient's permanent medical record. Drawn peripheral blood samples will be stored and a assigned a code, which will be identifiable only by the master list. The master list will be kept in a locked cabinet or a computer that would need a password to be accessed. Besides the PI and the key personnel, nobody will have access to the locked cabinet or the computer where the information is stored. The consent forms, authorization forms, and the coded index will be only available to the PI and key lab personnel involved in the project. The consent forms and authorization forms will be kept in a locked cabinet. The data will be kept on a secured server accessible only to the PI and key lab personnel involved in the project. The data recorded in the lab books and computer records will refer to the subject's code, not the name. Data shared with collaborators will be identified only by the code. The patients will never be identified in any publication.

When the results of the research are published or discussed in conferences, no information will be included that would reveal your identity.

9. Type and Number of Experimental Subjects

Subjects include patients over age 18, excluding pregnant women (self-reported), with an RA diagnosis, or other autoimmune disease to be used as a control. 200 donors will be treated with methotrexate, plaquenil and/or prednisone (DMARDs) that either achieve remission (DAS28<2.6) or do not achieve remission (DAS28>2.6). 50 donor will be utilized as they respond to DMARDs and achieve remission (DAS28<2.6) and 150 donors that do not respond to DMARDs will be treated with DMARDs such as methotrexate, plaquenil and/or prednisone and Cimzia provided to us by UCB. Subjects that fulfill the eligibility criteria will be identified by their physician/rheumatologist to be consented in participating in the study.

10. Description of Statistical Analysis to which the Data will be Subjected

For RA blood, using paired t tests, a sample size of 200 subjects will have a 80% power to detect the difference with effect size of 1.25 or above.

11. Potential Risks and Benefits to Subjects

The risks associated with blood draw include fainting, pain, a bruise at the site of vein puncture, inflammation of the vein. Every care will be taken to avoid these complications. The side effect of the drug Cimzia are similar to other 3 TNF-blockers these include serious infections, Invasive fungal infections, cases of lymphoma and other malignancies, Heart failure, Hepatitis B virus reactivation and Lupus-like syndromes.

Use of Cimzia may cause damage to the fetus. Therefore, it is recommended that patient practice abstinence.

Measures taken to minimize these risks are detailed above in #8. Samples and clinical information will be stored and cared for with every precaution taken to ensure confidentiality of participant information. There will be no compensation except for a parking sticker and testing results will not be released to individual subjects. However if the patients require to be treated with Cimzia (anti-TNFα), the medication will be provided to them free of charge up to 2 years. Subjects will not benefit directly, but receive medication.

12. Monitoring and Safety of Subjects

Possible adverse events that could be anticipated would be either complications arising from obtaining blood samples or breach of data security. While these events are unlikely, they would be immediately reported to the IRB. As above, all precautions will be taken to avoid adverse events, and any improvements in the sample collection and archival process that become available during the course of the study will be implemented (for example, new technology that allows the study of smaller amounts of blood sample, new widely available data security measures, etc.) Referring physicians will be charged with the continued monitoring of study participants as part of their routine care.

13. Procedures to Obtain and Record Informed Consent

Adult subjects will be provided with an informed consent and authorization form from study personnel, with full opportunity to ask questions and obtain clarification. This form will detail the study as described (please see consent form and authorization forms attached to this application). After description of the protocol, study staff will ask the subject to describe the study in their own words briefly to assess understanding of the key aspects, and any incomplete understanding will be addressed at that time also. All adult subjects will then receive an informed consent form if they wish to participate. The consent form will include phone numbers to contact study personnel if questions should arise in the future or if they should wish to discontinue their participation in the study. All original signed informed consent forms and authorization forms will be kept on file by Dr. Shahrara, and a copy will be given to the subject. Subjects wishing to discontinue their participation will have their samples removed from storage, however data collected or generated prior to withdrawal of consent may still be used in keeping with the above guidelines of the study (fully Coded without identifier, etc.).

14. Procedures which will be used to Maintain Confidentiality of Research and Study Subject Materials

A number of procedures will be used to maintain confidentiality of subjects. At enrollment, each subject will receive a designated study code number. All consent forms will be stored in locked file cabinets.

All personal health information will be stored in a secure database which only the PI and designated research staff can access. This database will be password-protected, and will be stored in a computer or in a locked file cabinet. Coded numbers will be generated for each subject, and these coded numbers will be used to label each specimen. These coded numbers will be used to file the subject's clinical information in an independent clinical database which is free of protected health information. The clinical database will also be password-protected and stored on a computer in a locked office. Only the PI and her designated staff will have access to the secure databases with protected health information and the link between anonymous specimen code numbers and patient identifying information. Coded data may be shared with other investigators or included in publications, but no patient identifying information will be released. Every precaution will be taken to ensure confidentiality of patient information.

15. Description of Recruiting Methods

No advertisements will be used. Patients will be recruited through their physician. If the patient is willing, then study personnel will discuss the study as described above in the informed consent section at a location and time that does not obstruct the clinical care of the potential subject or other patients in the outpatient area.

16. Description of how the Patient's Physician will be Notified or Involved in the Research

The patient's physician may be notified of whether the patient did or did not choose to participate in the study, if they are listed in the key personnel of the study and they obtain consent and authorization. There will not be any further involvement of the patient's

physician in the research after routine clinical care that provides the specimens. Clinical care will not be impacted in any way by participation or non-participation in this study.

17. References in the Text of the Narrative

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