Clinical and Immunologic Aspects of Rheumatoid Arthritis with a Focus on IL-6

Alan L. Epstein, MD: We are going to talk about some clinical as well as immunologic aspects of rheumatoid arthritis (RA). I think we are well aware of the importance of interleukin (IL)-6, tumor necrosis factor (TNF), and other proinflammatory cytokines, and the program is going to focus on IL-6 and TNF-α as well as other aspects of the immunopathogenesis of RA. I’m going to begin with some disease state discussion and then segue into some clinical aspects of the disease.

We’ve been discussing the issue of the shared epitope since the late 1980s. I very clearly remember chairing a symposium at the Four Seasons Hotel in Philadelphia; there were 400 participants, and we were talking about the shared epitope. We really did not know what we were talking about. We knew that such a construct existed, but we really didn’t know any detail about it. We now have a significant amount of information on what we mean by the shared epitope. So, what is it? In order to understand that concept, I need to review with you T-cell function.

Here, we have an antigen-presenting cell (APC). There is also a dendritic cell, it might be a macrophage, or it might be a B cell. The dendritic cell is picking up a protein antigen. It is going to process that protein antigen, as will be demonstrated in a moment, and a constituent peptide is going to be presented on the surface of the dendritic cell in the context of the major histocompatibility complex (MHC).

Looking again in some detail, there is an extracellular protein antigen. So, we are talking about proteins from the time when that protein antigen is going to be picked up in an endosome; it is going to be broken down into constituent peptides, and then one of those peptides is going to be presented out on the surface of the APC in the context of an MHC class II molecule.

Again, we have our APC presenting an antigen in the context of an MHC molecule trying to bind to a T-cell receptor and activate a T cell. If, in fact, the antigen is recognized by the T-cell receptor and it is the appropriate MHC molecule, the T-cell will be activated. If, on the other hand, we have the right antigen that could bind with the T-cell receptor but the wrong MHC molecule, there will be no recognition and no activation of that T cell. Furthermore, if we
have the correct MHC molecule for binding to that T-cell receptor—the MHC molecule is very important in terms of binding that T-cell receptor, it's not just the antigen—but the wrong antigen, binding and T-cell activation will also not occur. However, binding to the T-cell receptor alone is not adequate to activate the T cell; a second or costimulatory signal is needed, and there are a number of those. We talk a lot of the binding of CD80 or CD86 on the APC of CD28 on the T cell, which activates the T cell, but in fact there are a number of costimulatory signals. This is an incomplete list. For example, if CD40 on the APC binds to the CD40 ligand on the T cell, again the T cell is activated. On the other hand, if, for example, CD80 or 86 binds to cytotoxic T-lymphocyte-associated protein 4 (CTLA4) instead of CD28, there’s a down regulatory signal and inhibition of T-cell activation. So, there are numerous positive as well as negative costimulatory signals. Remember costimulation because it’s going to be important in a few minutes.

This has generated a three-signal hypothesis in terms of thinking about T-cell activation. Signal one is the antigen binding to the T-cell receptor in the context of MHC. The second signal is the costimulatory signal, for example CD80/CD86 to CD28, and then there’s a third signal that drives differentiation of the T cell. We’ll get to that in a moment.

Here’s a review: we have APC presenting the appropriate antigen in the context of the appropriate MHC molecule to the T-cell receptor; that’s signal one.

Signal two, where we have either CD80 or 86 binding to CD28, is a positive signal that will lead to T-cell activation.

Next is signal three. We have these T helper (Th)1 cells, Th2 cells, and Th17 cells; what does this all mean? When we begin with an undifferentiated T cell, the milieu in which that T cell finds itself is going to determine whether that undifferentiated T cell becomes a Th1 cell—a group that is important to RA—or a Th2 cell, which a little less important in RA, or an extremely important subset, Th17. They’re very important, very inflammatory cells. The Th17 cells produce, amongst other cytokines, IL-17, a very important proinflammatory cytokine. In addition, you will notice the importance of IL-6 in leading to the differentiation of the undifferentiated T cell into this very important proinflammatory Th17 cell, or the fourth category, the more recently recognized, regulatory T cells. So, the cytokine milieu in which the T cell finds itself will determine whether it becomes a Th1, Th2, Th17, or regulatory T cell. That cytokine milieu is largely, though not entirely, determined by this APC.

Let’s talk a little bit about factors that predispose the development of RA. One
would clearly be genetics, and there will be more to say about that in a moment. The \textit{HLA-DRB1} gene is an important risk factor for RA; another one that is spoken less about is \textit{PTPN22}, which is a gene that codes for a protein that is involved in B- and T-cell regulation. It is known that the environment is important. Researchers have been looking for the triggering infection for decades. I remember back in 1983, the woman in the lab next to mine thought that she had discovered the virus that caused RA; she called it the RA1 virus, and it was the hit of the national meeting in 83-84. We know how many publications have come from that: none. So, the community is still looking for that presumed infection that is important in triggering RA, but it has not yet been found. It is known that hormones are important, and that women are more prone than men to diseases like RA. The importance of smoking is well known, as well as the fact that there’s a dose-response relationship: the more you smoke, the more your risk of RA. It is well known that smokers respond less well to TNF inhibition in RA, so there are important environmental factors. And then autoimmunity is a factor as well.

Th1 and Th17 cells are important. A couple of decades ago, RA was thought of as a T cell-driven disease. So, this was an adaptive immune disease. Well, let’s debunk that myth right away. Not too long ago, perhaps 10 or 15 years ago, a researcher in England demonstrated that a depletion of B cells ameliorated RA, so now B cells have become important in this so-called T cell-driven disease. Well, what about innate immunity? The APC, which is part of the innate immune system, is important as well. I’m here to tell you that RA is not just a T-cell disease—the B cells are important too—it’s not just an adaptive immune disease. Innate immunity is important too, and by the way, that is true of many of our diseases. For example, lupus is a B cell-driven disease; however, the pathogenic autoantibodies in lupus are high-affinity immunoglobulin G (IgG) autoantibodies, except our B cells are inclined to produce low-affinity immunoglobulin M (IgM) autoantibodies. So, the way you get from a low-affinity IgM to a high-affinity IgG is with T cell help. So in fact, lupus is not just a B-cell disease, it’s also a T-cell disease.

Let’s talk a little more about the genetics of RA and where that risk related to the \textit{HLA-DRB1} locus comes in. The \textit{HLA-DRB1} locus codes for an HLA class II beta chain molecule that’s part of that MHC, which I’m going to show you in a minute.

What is tolerance? The risk of developing RA is pulled together with a few aspects of immunology. The definition of tolerance is the specific unresponsiveness to an antigen. Why is it important? We’re all tolerant of our own antigens, so-called self-tolerance. The breakdown of that self-tolerance is what results in autoimmunity. In order to develop RA, there needs to be a break in tolerance. There are a couple of different types of tolerance: so-called central
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tolerance and peripheral tolerance. So, with central tolerance, we have some immature lymphocytes that are self-reactive lymphocytes; they’re going to react to self antigens, and here we’re talking about the thymus and the bone marrow. So, there are these immature lymphocytes that are able to recognize self-antigen either in the thymus or the bone marrow, depending on whether discussing B cells or T cells. Well, a few things can happen. Number one, that highly self-reactive T cell can simply be deleted by apoptosis. If it is a B cell, that B cell may simply change its receptor affinity, so-called receptor editing, or if it’s a T cell, it may develop into a regulatory T cell, which we’ll look at in a minute.

What about peripheral tolerance? We have the APC presenting antigen to the T cell, and the T cell is being activated. In the periphery, there’s recognition of the fact that the cell is self-reactive; it may simply become functionally unresponsive due to the lack of costimulation. So, when there’s no costimulation, the cell is functionally unresponsive; that second signal is needed. It may be deleted if it’s a self-reactive cell, or it may be suppressed by a regulatory T cell. There are a number of mechanisms by which a self-reactive T cell in the periphery can be removed. It can be removed by anergy, simply lacking that secondary signal and being unresponsive; it may simply be deleted; or it may be controlled by regulatory T cells.

Now the question is, how is tolerance breached in RA?. There is one last concept that needs to be covered, and that is the concept of citrullination. First of all, we all citrullinate all the time. Citrullination is simply the conversion of an arginine residue into a citrulline residue, and we’re all doing this all the time. Although there are some people who believe they know why citrullination occurs, in reality we’re really not sure why it occurs in a normal person. What happens is at many sites of inflammation, neutrophils release this enzyme called peptidyl arginine deiminase (PAD), and that is the enzyme that citrullinates proteins. Let’s look at that in some detail. Here, we have an arginine residue; notice it’s positively charged, and due to the involvement of PAD, that arginine becomes a citrulline. Notice a few things: the positively charged arginine moiety is now a neutral citrulline. Secondly, when the charge is changed, the tertiary structure of the protein changes. Now what used to be a wound-up smaller protein is a larger unwound protein. And thirdly, some epitopes that were previously not visible to the environment were unmasked. So, three things: 1) the charge has changed; 2) the size of the protein has changed; and 3) some new epitopes have been unmasked. What does that mean?

Here, we have the APC, and here we have the MHC class II molecule, the alpha chain and beta chain, which is coded for by the HLA-DRB1 locus. Here is the binding site for that peptide. Remember, that peptide was processed and
presented on the surface of the APC in the context of MHC. Now I don’t have RA. If this is my MHC molecule, the binding site is too small to accommodate that larger, differently charged citrullinated residue. This is the key to understanding the genetics of RA, at least as we understand it today. The patient with RA has a larger binding site, and that is because of the shared epitope in that binding site. This leads to a larger binding site that can accommodate that citrullinated protein, which in my binding site is too small to accommodate. Now the self-protein has been citrullinated. It’s still self-protein and it’s been presented to one’s own MHC molecule; it’s being presented to the T cell but recognized as foreign, so a patient’s tolerance is breached and overcome. The patient’s immune system is now recognizing their own protein as foreign and activating T cells. The T cells are helper T cells, so they’re activating the B cells, and the B cells are producing autoantibodies and driving the process.

That’s looked at here. We recognize that RA is a complex disease; it involves environment and genetics as we just talked about, and it involves innate and adaptive immunity. It also involves a number of different cell types including B cells, T cells, macrophages, dendritic cells, fibroblast like synoviocytes—these are beyond the time we have today—but it also involves a multitude of cytokines: IL-1, IL-6, and TNF to drive this very complex disease.

The question comes up, is the glass half empty or half full? The treatments we had prior to 1998 were developed by mistake. So, how was methotrexate developed for RA or discovered for RA? Well, dermatologists were giving their patients methotrexate for their skin, and said “hmm…their joints are getting better.” Back in the mid-1980s, rheumatologists didn’t have great treatment for RA, so we said, “Well, if it helps psoriatic arthritis, let’s try it in RA.” Then it became the mainstay of our armamentarium, but discovered fortuitously. Now, because we have such a greatly improved understanding of the immunopathogenesis of RA, we can now talk about targeted therapies. We can target TNF and see if it works; it does. We can target IL-6 and see if it works, and I’ll show you it does. We can target IL-17 and see if it works, and it didn’t in RA, which is confusing, but obviously the body has some way of bypassing IL-17 blockade in RA because that inhibition didn’t help. It doesn’t mean that IL-17 isn’t important; it just means that inhibition didn’t help. So, we can intervene on a number of different targets. We’re well aware of the fact that we can deplete B cells and help our patients, we can block T-cell activation and help our patients, we can block TNF-α and help our patients, and we can actually block IL-1 and help our patients. We don’t do that much anymore of course. The focus right now is what happens if one blocks IL-6.

So, the significance of IL-6 as an important cytokine in RA has been recognized.
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Here’s some data looking at levels of IL-6, IL-1, TNF, and IL-8 in the serum and the synovial fluid. You can see elevated levels of all of those proinflammatory cytokines in patients with RA. If we compare the level in the synovial fluid in patients with osteoarthritis (OA) and RA, you can see clearly more IL-6 in those with RA compared with patients with OA.

I think a lot of rheumatologists are “scared,” if you will, by the potential toxicities of IL-6 blockade. The truth is, if you understand the mechanisms of IL-6 and IL-6 blockade, the supposed toxicities are predictable, understandable, and surmountable. For example, first of all, IL-6 comes from a lot of different sources: macrophages, B cells, T cells, fibroblasts; multiple different cells produce IL-6. Look at what IL-6 does; it promotes platelet production. So, it’s understandable that if one blocks platelet production with IL-6 blockade, one is going to see, potentially, a drop in platelet count; it’s predictable. If one blocks IL-6, one can see a drop in white count, also predictable. IL-6 also drives B-cell differentiation in immunoglobulin production. It drives T-cell differentiation as well, so this is a cytokine that comes from many sources and has many effects on the body.

This is the importance of IL-6 in terms of T-cell differentiation. You might remember that naïve T cell, when it’s in a cytokine milieu including transforming growth factor (TGF)-β and IL-6, will differentiate into a Th17 cell, a very proinflammatory subset of cells. On the other hand, the presence of IL-6 actually blocks the development of regulatory T cells. So, IL-6 is very important in terms of T-cell differentiation.

Well, cytokines need to signal, and IL-6 has some somewhat unique signaling properties, which I’m going to review with you now. Here, we have IL-6, and a relatively small number of cells in our body have an IL-6 receptor on their cell surface, including some hepatocytes, T cells, B cells, and macrophages. With that as the case, IL-6 can bind to the IL-6 receptor. That complex then engages glycoprotein 130 (gp130) leading to intracellular signaling, which I’ll show you in a minute. By the way, that’s referred to as either classic signaling or cis-signaling. There’s another type of signaling referred to as trans-signaling. Here, we have IL-6 binding to a soluble, now no longer membrane-bound, IL-6 receptor, whose complex can then bind to gp130. The pp130 is quite ubiquitous, found in many, if not most, cells.

Then, we can look at how signaling continues. Here, we have the IL-6 receptor, either membrane-bound or soluble, now binding to the gp130 receptor. When that interaction takes place, there is a transformational, conformational change in the gp130 receptor where these two Janus kinase molecules come together and they autophosphorylate each other. When they are phosphorylated, they’re
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activated. When that happens, they in turn phosphorylate the gp130 molecule. When that happens, signal transduction and transcription (STAT) protein is able to bind. When STAT binds, it is in turn phosphorylated. It can then dimerize, translocate to the nucleus, and lead to the transcription of various proinflammatory factors, as well as factors such as matrix metalloproteinases that lead to cartilage degradation or receptor activator of nuclear factor κ-B ligand (RANKL) that leads to bone erosion.

In fact, our currently available drugs don’t block IL-6 itself, but rather they block the IL-6 receptor. We’re familiar with tocilizumab; we have that available now, and during the next few minutes, I’m going to introduce you to sarilumab, another IL-6 receptor blocker that is currently being reviewed by the US Food and Drug Administration (FDA) for approval. You can see these monoclonal antibodies binding to the IL-6 receptor to prevent binding of IL-6 to its receptor.

Realize that when RA begins, it begins as an acute inflammatory process. So, inflammatory stimuli recruit neutrophils from the blood, and they come into this acute inflammatory focus. If that lasts for very long, the organism is going to be destroyed. There has to be a way to change from an acute inflammatory event to a more chronic inflammatory event such as RA. IL-6 is important in that transformation, as I’m going to describe to you now. So, the activation of neutrophils and proinflammatory signals stimulate proinflammatory cytokine production. These various proinflammatory factors lead to the apoptosis of neutrophils; I’ll show you a diagram in a second. When the neutrophils die, they release IL-6 receptors. Those IL-6 receptors that are released can then bind IL-6 in the joints. So, now there’s a complex of an IL-6 receptor with IL-6, and when that occurs in an endothelial cell, there’s a production of monocyte chemoattractant protein 1, or monocyte chemotactic protein 1 (MCP-1), which brings monocytes in where neutrophils previously were.

So that’s shown here a little more clearly. Here we have a very proinflammatory situation, which leads to the apoptosis of these neutrophils. The neutrophil sheds IL-6 receptor and the IL-6 receptor binds to the IL-6 present in the joint creating an immune complex of IL-6 receptor and IL-6. This binds, as I just showed you, to gp130, and if that is in the context of the endothelial cell, the endothelial cell produces MCP. Now a patient is going to get the influx of monocytes we’re talking about into the joint, and the patient has converted from acute inflammation to the chronic inflammation characteristic of RA.

I hope that has been clear, and what I’m going to do now is to transition into some technical data.
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So, tocilizumab we’re familiar with; it’s a humanized inhibitor of IL-6 activity. As I said, it inhibits IL-6 receptors both on the membrane surface as well as the soluble IL-6 receptor. It can be administered either as monotherapy or in combination with methotrexate or other nonbiologic disease-modifying drugs, and it’s available for intravenous (IV) or subcutaneous (subQ) administration.

Let me share with you one clinical trial briefly. The SUMMACTA trial was a study that looked at the safety and efficacy of tocilizumab subQ compared with tocilizumab IV in combination with traditional disease-modifying antirheumatic drugs (DMARDs). This was a noninferiority trial to demonstrate that subQ tocilizumab was noninferior IV tocilizumab, and the primary endpoint was the American College of Rheumatology 20 (ACR20) at week 24.

The data shows the following: the ACR20, 50, and 70, are robust numbers. You can also see that the subQ and the IV responses were superimposable, so clearly the noninferiority endpoint was met.

Let’s discuss sarilumab. Sarilumab is a fully human antibody against the IL-6 receptor; it’s a monoclonal antibody that binds both the membrane-bound receptor and the soluble receptor and does so with high affinity. It blocks both the classic signaling, the membrane-bound signaling called the cis-signaling, and the soluble receptor, so the trans-signaling.

One of the studies that was presented to the FDA that is being considered for the approval of this drug is the MOBILITY trial. The MOBILITY trial was a multicenter, randomized, double-blind, placebo-controlled phase III trial. The patients were incomplete responders to methotrexate and had well-established disease. This is not early RA; these patients had on average nine years of disease, and they had very active disease. They had approximately 27 tender and 17 swollen joints, and they had to be either seropositive or had to have erosive disease—poor prognostic signs. Patients were on background methotrexate therapy and were randomized to placebo, sarilumab 150 mg, or sarilumab 200 mg given every other week.

Here are the data. You can see a nice robust primary endpoint in the ACR 20, 50, and 70, and response was well maintained at week 52. This shows good efficacy.

We always want to talk about the ability of the drug to inhibit erosive disease. You can see almost complete inhibition of radiographic progression over the one year that this trial was conducted.
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Well, what about safety? Severe adverse events (SAEs) were somewhat elevated; so 5% of patients in the methotrexate had SAEs, and there was a slight elevation in SAEs with the sarilumab (9-11%). If you look at some other toxicities a little more clearly, and we can look at infections, there were about 30% with the methotrexate alone and 40% in the sarilumab groups. When we think about blood disorders and things like neutropenia, again, predictable neutropenia, that’s how the IL-6 stimulates white cell production. Neutropenia can be blocked and predicted, and, again, there was some neutropenia. There were some abnormal liver function tests for example, but generally these changes did not lead to withdrawal of the drug.

I want to address with you the question about whether or not disease-modifying therapy can be withdrawn in patients who are doing well. The truth is, we’ve been using these drugs for 15 years now, and I think most of us, if we can get a patient with RA on methotrexate along with a biologic and they do well, we’re thrilled. We don’t really think that much about whether or not we can withdraw the biologic therapies. Can we do so and maintain efficacy? If we do so, perhaps we can improve safety and certainly reduce costs. So, there is good reason why we might want to try to withdraw a biologic therapy. There are three trials of importance in this area. The first is the OPTIMA trial that was done with adalimumab. It was a multi-armed trial that began with adalimumab plus methotrexate compared with methotrexate alone. It was actually a treat-to-target trial, and I’m not going to get to the treat-to-target part, unless you’d like me to in terms of Q&A. If a patient achieved low disease activity at week 22 and week 26, they were re-randomized to either continue on the combination or drop the adalimumab—withdraw the adalimumab completely to see if their response could be maintained. The data demonstrated that a lower-level endpoint like an ACR20 can be maintained. You can see that ACR20 is maintained. However, the more robust endpoint, ACR70, is not maintained by the lower dose. There is in fact a significant difference between continuing adalimumab and dropping it. Although the patients are doing fairly well, we’re not saying they’re doing terribly, there is a significant difference in terms of whether the adalimumab is dropped completely or maintained.

So, again, maintenance of low-disease activity was greater with the combination group, but the methotrexate alone group did reasonably well. I think that’s a fair statement, although there was a significant difference.

Well, how about etanercept? These data were from the PRESERVE trial, which was interesting in that our typical studies take patients who have high-disease activity. Here, the patients were enrolled with moderately active RA, which is
arguably more typical to what we are used to seeing in our offices. So, they got treated in a standard way with standard etanercept plus methotrexate. If they achieved low disease activity at 36 weeks, they were re-randomized to three groups. One group continued standard therapy. The second group got half-dose etanercept, 25 mg instead of 50 mg, and in the third group the etanercept was dropped completely.

Whether you look at low disease activity on the Disease Activity Score (DAS), remission, low disease on the Simplified Disease Activity Index (SDAI), or SDAI remission, the conclusion is the same. That is, the dose of etanercept can be reduced but not stopped. When we stop and maintain patient just on methotrexate, we see a very significant reduction in disease control. So, again, the dose of etanercept could be reduced, but if it was dropped, the patient lost disease control significantly.

The same was shown in this study with infliximab. The study included a small number of patients in whom the infliximab dose was slowly reduced. What they found was that they could stop infliximab in 16% of patients and reduce the dose but not stop in 45% of patients. In 39% of patients, when they tried to do that, the disease flared. So, I think what we may be thinking is that we may be able to reduce the dose of biologic therapies, at least in terms of the TNFs that we’re looking at now, but not completely stop.

The final study I want to show you is the CONCERTO trial. This was actually done to look at the safety of methotrexate when given with adalimumab. There was another aspect that I want to share with you, and here’s how it was designed: patients either received 2.5 mg, 5 mg, 10 mg, or 20 mg of methotrexate plus adalimumab. I think most of you do a similar thing to what I do; we start somebody with seropositive RA on methotrexate, and we start them at maybe 10 mg for 3 or 4 weeks and then up to 15 mg for 3 or 4 weeks. We then go up 20 mg, and if they’re not responding adequately, we’re going to add a biologic. So, what then do we do with the methotrexate? Now the patient responds to the biologic, and I think a lot of us say, “Well, they’re on 20 mg of methotrexate and a biologic and they’re doing well. We’ll sit tight.”

Well, if in fact you drop the methotrexate from 20 mg down to 10 mg, the efficacy is the same, at least when used with adalimumab. So, what you might consider doing in that patient who’s doing well on combination therapy is not stop methotrexate, but just reduce it down to 10 mg. A lower dose is safer presumably.

Again, is the glass half-empty or is the glass half-full? Autoimmune diseases
such as RA pose an increasing, worldwide economic and health burden. We don't have a cure at this time. Our treatment is aimed at controlling symptoms and preventing progression and disability. Remember that not too long ago, before we had these biologics, 40% to 80% of patients with RA were disabled at 10 years. So, when we used what we used to call disease-modifying drugs like gold and penicillamine, and even methotrexate, those patients were commonly disabled at 10 years. We now have developed new treatment approaches, but we don't have a complete understanding yet of the immunopathogenesis of the disease. As we better understand the mechanisms of the disease, we'll be perhaps allowed to have predisease diagnosis; we could stratify better, and we could have more specific therapies.