Rheumatoid Arthritis

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Rheumatoid Arthritis (RA) is an autoimmune disease that affects 0.5-1% of adults in industrialized countries and is caused largely by genetic factors. The study of this disease is important to those suffering from the condition and to decrease subsequent generations from suffering. At this time, RA patients are suffering with limited options available to them. Long term effects from current treatments have not been studied enough to have conclusive results. Although the exact pathogenesis of this disease has not been determined, there are numerous studies done on the different aspects of the disease. Studies done on Rheumatoid Arthritis include angiogenesis, anxiety and inflammation, as well as methylation patterns in the genomes of patients with Rheumatoid Arthritis.

Rheumatoid Arthritis causes severe inflammation and progressive joint destruction. Research has shown that cytokines, due to their cell signaling capabilities, play a significant role in the joint destruction of Rheumatoid Arthritis (RA) affected patients. Cytokines TNF-α, interleukin (IL)-1, and IL-6 have been identified as active components of the disease (Hillyer, 2009). The interleukins and cytokines are found in especially high levels in the synovial fluid of RA patients causing inflammation. The synovial membrane is a thin layer of tissue surrounding joints and it secretes the synovial fluid that helps lubricate the joint. Research has been directed towards cytokines for therapy treatments in order to help patients control their inflammation. More recently, evidence has found that excessive levels of interleukin IL-23 in RA patients influences joint inflammation and bone destruction. Researchers are looking to target interleukin-23 and reduce its quantity in order to help lessen the destructive nature of the disease.
Rheumatoid factor has been identified as the autoantibody present in RA patients and useful for categorizing patients into specific treatment plans. Citrullinated peptides are peptides that have been damaged or modified and cause an autoimmune response. The enzyme peptidylarginine-deiminase (PAD) in the presence of calcium, converts the amino acid arginine into the amino acid citrulline. Once these citrullinated peptides are present, the body creates anticitrullinated peptide antibodies that initiate inflammation (Citrullination, 2013). Citrullinated peptides are almost exclusive to Rheumatoid Arthritis patients but not all patients with Rheumatoid Arthritis have citrullinated and anticitrullinated peptide antibodies present (Scott, 2010).

Interleukin-26 is a particular cytokine that is part of the production of the inflammatory response in epithelial cells. The role of Interleukin-26 was analyzed in order to determine its significance in the autoimmune response in Rheumatoid Arthritis. They used the ELISA method in order to quantify the Interleukin-26 concentrations comparing Rheumatoid Arthritis patients, other arthritis patients, as well as healthy subjects (Corvaisier, 2012). This type of method is used to screen for particular proteins and quantify an antigen in a sample. The surface of an assay is covered in antibodies against the protein interleukin-26. The interleukin-26 is then immobilized on the assay surface. An additional antibody is added that has an enzyme linked to it, so when the antibody reacts with the antigen a secondary reaction will take place with the enzyme when a substrate for the enzyme is added. This secondary reaction displays a visible signal such as a color indicating the antigen is present.

This study compared the serum and synovial fluid between 26 healthy individuals, 22 Rheumatoid Arthritis patients and 13 others with inflammatory diseases. These researchers were able to conclude there is a large difference between Interleukin-26 levels in patients with arthritis, both RA patients and other patients suffering from arthritis, and the levels of Interleukin-26 in healthy
individuals. In healthy individuals IL-26 levels were about 0.01ng/mL and in Rheumatoid Arthritis levels were approximately 5ng/mL. This study also demonstrated there were significantly higher levels of Interleukin-26 in the synovial fluid when compared to the serum. In the synovial fluid, levels of IL-26 were even greater, with an average around 55ng/mL but slightly less than other arthritis patients who averaged around 60ng/mL. With this data, researches sought the production site of Interleukin-26 in the synovial fluid (Corvaisier, 2012).

Immunohistochemical staining revealed Interleukin-26 was expressed heavily in the hyperplastic lining cell layer in the synovial membrane of Rheumatoid Arthritis patients. Hyperplasia is typically demonstrated by a thickening of cell growth, often precancerous. Immunofluorescence staining further disclosed synoviolar fibroblast-like synoviocytes and CD68+ macrophage-like synoviocytes expressed Interleukin-26 within this region of the hyperplastic lining cell layer. This study also demonstrated how Interleukin-26 was upregulated by Interleukin-1-beta and Interleukin-17A, both of which have been shown to be a part of the pathogenesis of Rheumatoid Arthritis. This was done by isolating CD14+ myeloid cells with Interleukin-26 and monitoring the production of Interleukin-1-beta, Interleukin-6 and TNF-alpha production in both healthy and Rheumatoid Arthritis patients. CD14+ myeloid cells were chosen because they are responsible for controlling the inflammatory process of T cells. Furthermore, they were able to characterize the specificity of Interleukin-26 by fluorescence-activated cell sorting (FACS) which detected the presence of Th17/ and Th22 cells in memory CD4+ T cells when subjected to Interleukin-26. The results showed Interleukin-26 enhanced the frequency of Interleukin-17A-producing T cells. When the cells were stimulated with IL-1beta, averages rose from about 0.3ng/mL to about 0.9 ng/mL for IL-26. This is significant compared to the effect IL-1beta had on IL-26 levels in healthy subjects. In healthy subjects there was about an 0.005ng/mL difference in IL-26 levels when adding IL-1beta, and without it. IL-17A also created about a 0.3ng/mL difference in IL-
26 levels in Rheumatoid Arthritis patients compared to the negligible difference in healthy patients. When the cells were saturated with IL-1beta and IL-17A there was the greatest overall difference in Rheumatoid Arthritis patients with an increase of 0.8ng/mL compared to 0.05ng/mL in healthy patients. Overall this study clarified the role of Interleukin-26 in Rheumatoid Arthritis patients and its specificity to RA synoviocytes in inflamed joints (Corvaisier, 2012).

Interleukin-6 is known to be a key part of angiogenesis in Rheumatoid Arthritis patients. Angiogenesis is a known symptom of Rheumatoid Arthritis and is stimulated by vascular endothelial growth factor (VEGF). In this study, cells from the synovial fluid of Rheumatoid Arthritis patients as well as human umbilical vein endothelial cells were grown. After a few days, the cytokines Interleukin-6, VEGF or Ang-1 were added. After six days, the growth was observed and they compared the production of cells in Rheumatoid Arthritis patients and the human umbilical vein endothelial cells. Results showed Interleukin-6 and VEGF both enhanced the cell growth but the morphology of the cells produced in the presence of Interleukin-6 were much more scattered compared to those produced by VEGF. Angiogenesis is stimulated by Ang-1 and is countered by Ang-2. Ang-1 and Ang-2 concentrations were analyzed by ELISA in cultures containing Interleukin-6, VEGF, or without both. In cells subjected to IL-6 Ang-1 was about 35 picogram/mL, Ang-2 picogram/mL was about 5000 picogram/mL, and VEGF levels were about 80pg/mL. This was compares to the levels in cells stimulated with VEGF: Ang-1 levels about 90pg/mL and Ang-2 levels about 6000pg/mL. Results showed Ang-1/Ang-2 ratio was lower in cultures with Interleukin-6, about 0.2, than in VEGF, about 0.35, concluding that VEGF was shown to be at higher levels with Interleukin-6 present (Kayakabe, 2012).

Overall the data suggests that Interleukin-6 does increase angiogenesis but in a destabilized manner. Interleukin-6 inhibited the production of Ang-1 but increased the production of Ang-2.
Interleukin-6 shows an increase of VEGF production and a lowering of Ang-1 production. Angiogenesis is increased in Rheumatoid Arthritis patients and enhances the inflammation in the joints. Angiogenesis causes an accumulation of leukocytes into the synovium of Rheumatoid Arthritis patients further stimulating inflammation to occur in the joints. VEGF and Angs are important factors of angiogenesis and a better understanding of these mechanisms will help find therapeutic medicines to reduce angiogenesis, consequently helping reduce inflammation (Kayakabe, 2012).

Recent studies were done to determine if Rheumatoid Arthritis patients have an increased level of the cytokines TNF-α, IL-6, and IL-17 and if they correlate with depression and anxiety. Eighteen patients effected with Rheumatoid Arthritis and eighteen healthy patients, as a control, were tested for their level of anxiety, depression and pain levels. The subjects were then tested for their cytokine levels by the use of the Enzyme-linked Immunosorbent Assay method (ELISA). This type of method is used to screen for particular proteins and quantify an antigen in a sample. The surface of the assay is covered with an antibody against the proteins of interest, in this case the cytokines TNF-α, IL-6, and IL-17. Analysis revealed high Interleukin-17 levels were associated with high anxiety in the entire population, both RA patients and the control group. There was a relation with Interleukin-17 levels and anxiety levels unlike with the other cytokines TNF-a and IL-6. This is critical because Interleukin-17 plays a key role in the inflammatory response of Rheumatoid Arthritis patients. In order to control the inflammatory response of Rheumatoid Arthritis patients, anxiety levels should be lowered in order to keep Interleukin-17 levels maintained.

Interleukin-23 is another cytokine that was determined to have an important role in Rheumatoid Arthritis patients. A study was to determine the effects of Interleukin-23 on the inflammation response in patients affected by the disease. Cultures were produced from the synovial fluid supernatants and the cell lysates of Rheumatoid Arthritis patients. The cultures were subjected to the presence or absence of
antibodies against Interleukin-23, Interleukin-12, Interleukin-17, and levels of TNF-a, Interleukin-6 and Interleukin-1B were measured by ELISA. Their expression levels were later determined by PCR. ELISA detected especially low levels of Interleukin-23 compared to the other interleukins from the supernatant fluid of the cultures yet found a much higher level from the cell lysates. These researchers of this study concluded that Interleukin-23 must be surface bound in cells because of the high numbers found in cell lysates than that found in the secretions (supernatants).

In order to determine whether Interleukin-23 had an effect in the inflammatory response of Rheumatoid Arthritis patients, antibodies for Interleukin-23 were flooded in Rheumatoid Arthritis synovial cultures and examined what cytokines were further produced from the culture. The antibodies inhibited TNF-a mRNA from being produced, about 73% and Interleukin-1B mRNA, by about 88%. These two proteins are part of the inflammatory process of Rheumatoid Arthritis and this information could be used for therapeutic treatments to reduce inflammation using these antibodies.

Methylation studies were done on Rheumatoid Arthritis in order to help clarify the genetic aspect of this disorder and to find if diagnosis could be done through a patient's genome. This study compared the DNA genomes of synovial fluid from Rheumatoid Arthritis patients, Osteoarthritis patients, and healthy controls. The researchers of this study chose to focus on miR-124a because this gene is known to be downregulated in Rheumatoid Arthritis synovial tissues and hypermethylation is known to cause gene silencing. The genomes of the RA patients, OA patients, and healthy control patients were extracted and quantitated by methylation-specific PCR. Methylation-specific PCR (MSP) was used to determine the methylation status of the genome by amplifying the DNA and using primers, taq polymerase, and an unmethylated control. MSP is used to amplify specific, heavily methylated regions of interest. Methylated-specific primers on bisulfite-converted DNA is used to anneal to methylated regions only. They then compared the genomes against the controls and against the different
The results showed that the miR-124a was methylated 57.1%-71.4% of the time in Rheumatoid Arthritis tissues, 0-16.7% in osteoarthritis patients, and 0% shown in the healthy individuals. This study shows that the down regulation of the miR-124a genes could potentially be because of the hypermethylation at this position. The field of gene therapy is rapidly expanding and this information provides the opportunity for further research in miR-124a as a possible target for demethylation for a potential therapy (Zhou, 2013).

In conclusion studies have been done to determine the different roles proteins play in Rheumatoid Arthritis. Although a definitive mechanism has not yet been discovered, researchers have narrowed down to several interleukins and cytokines and their roles in the disease. High levels of Interleukin-23 have been found to cause joint inflammation and bone destruction in Rheumatoid Arthritis patients and that accumulation of this interleukin is found most prominent in synovial fluid. Interleukin-6 is also understood to be a key component of angiogenesis in patients with Rheumatoid Arthritis. In the studies done correlating anxiety with inflammatory symptoms, Interleukin-17 was determined to play a key role. Studies done on interleukin-23 also showed its vital impact on subsequent proteins being produced and their overall impact on inflammation in patients with Rheumatoid Arthritis. Methylation studies showed a gene methylated more commonly in Rheumatoid Arthritis patients than other healthy patient types. The more research that is done the more opportunities available for therapies to be made. Their are numerous facets to this disease and with all the proteins involved, the more research done on individual proteins, the closer we become to a treatment!
Works Cited


