Targeting a novel pathogenic neutrophil subset in SLE

Arthritis Australia Project Grant- Australian Rheumatology Association (ARA)

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Scientific Report

Background

Systemic lupus erythematosus (SLE) is an autoimmune disease with a wide spectrum of clinical features. In this disease, chronic inflammation causes organ damage, with poor outcomes for patients. Despite decades of research, we still do not fully understand what causes this or how best to treat it. Furthermore, biomarkers that predict disease activity are severely lacking. While research has predominantly focused on T and B cells, it is becoming increasingly clear that neutrophils play an important role in SLE. While essential for host defence, dysregulated neutrophil responses contribute to the pathogenesis of SLE. Neutrophils become hyperactive in SLE, resulting in the release of proinflammatory molecules and mediators of tissue damage. Formation of neutrophil extracellular traps (NETs) is enhanced in SLE, leading to autoantigen exposure, and inciting the production of autoantibodies. NETs also directly promote tissue damage. While neutrophils were long considered a homogeneous population, it is now recognised that pathogenic subsets can appear in disease. Using single cell profiling, we have identified a novel neutrophil subset in SLE**,** which is absent or minimal in healthy people. This subset is defined by expression of CD98, an essential amino acid transporter. CD98 is responsible for importing essential amino acid into cells, which in turn fuels the mitochondria to produce energy. CD98 neutrophils are more metabolically active, which amplifies their pathogenic capacity. Our preliminary data suggests a correlation between CD98 neutrophils and disease activity in SLE, potentially providing a new biomarker.

Hypothesis

We hypothesize that CD98 neutrophils are pathogenic and targeting this subset may prevent neutrophil-mediated inflammation and organ damage in SLE. We also hypothesize that the CD98 neutrophil population represents a novel clinical biomarker for SLE.

Grant objectives and aims:

Our overall goal was to decipher the role of CD98 neutrophils in SLE. In this project funded by Arthritis Australis, we addressed the following aims:

Aim 1: Validate CD98 neutrophils as a novel biomarker in SLE.

Aim 2: Target CD98 on neutrophils to limit disease in a preclinical model of SLE.

Results

Aim 1: LDN are expanded in PB of SLE patients and are pathogenic.

We sought to determine if CD98 neutrophils could be used a prognostic biomarker to indicate disease activity in SLE. For this study, patients with active disease were recruited through the Rheumatology Unit at the Royal Melbourne Hospital and healthy control donors were recruited through the Volunteer Blood Donor Registry at WEHI. Peripheral blood was collected and the number of CD98 neutrophils was quantified using flow cytometry. Very few CD98 neutrophils were detected in the blood of healthy individuals, while SLE patients had increased numbers of this subset. Strikingly, the number of CD98 neutrophils in peripheral blood of SLE correlated with disease activity scores as measured by SLEDAI-2K. This suggests that quantification of CD98 neutrophils in peripheral blood could serve as a novel prognostic biomarker to measure disease activity, remission or response to treatment and assist with clinical management of SLE patients.

We conducted further analysis of the CD98 neutrophils compared to normal neutrophils that do not express CD98. We assessed size, granularity, and the maturation status of the two neutrophil populations in SLE using flow cytometry. CD98 neutrophils were comparable in size, but more granular than normal neutrophils, as indicated by an increase in mean side scatter. This is in keeping with our observation that CD98 neutrophils express more granule proteins including elastase, proteinase 3 and myeloperoxidase, all known to cause endothelial and tissue damage in autoimmune diseases. CD98 neutrophils were comprised of both mature and immature neutrophils, as determined by CD10 expression. Functionally, CD98 neutrophils were resistant to cell death and capable of generating more proinflammatory cytokines and chemokines, especially IL6, with a 40-fold increase above baseline. Lastly, our results suggest that the pathogenic capacity of this neutrophil subset may be directly due the expression of CD98. CD98 neutrophils were more energetic and produced energy through

both glycolytic and mitochondrial dependent pathways, whereas normal neutrophils generate energy through glycolysis only. Using a potent CD98 inhibitor (JPH203), we demonstrated that CD98 is directly responsible for fuelling the increased bioenergetic capacity in this neutrophil subset (Fig 2D). In these experiments, JPH203 inhibited CD98 mediated accumulation of amino acids that are essential for powering mitochondrial energy production, which in turn may facilitate their proinflammatory function.

Aim 2: CD98 neutrophils are present in a highly relevant animal model of SLE.

To evaluate whether CD98 neutrophils contribute to the pathogenesis of SLE *in vivo*, we used the Lyn-deficient ($Lyn^{-/-}$) mouse model. This is a well described experimental model of SLE that develops the serological and pathological hallmarks of the disease including autoantibody production, inflammation, and glomerulonephritis. Activation of the autoimmune response can be observed as early as 12 weeks and mice develop severe disease and renal damage by 36 weeks. Given CD98 neutrophils are found in SLE patients but not healthy people, we first evaluated levels of this neutrophil subset in $Lyn^{-/-}$ mice. Cohorts of $Lyn^{-/-}$ mice with mild (12 weeks), moderate (24 weeks) or severe (36 weeks) disease were evaluated. Age matched wild type control mice were also assessed at each timepoint. While CD98 neutrophils were present at similar levels in the bone marrow of wild type and $Lyn^{-/-}$ mice at all timepoints examined, wild type control mice had very few CD98 neutrophils within the spleen or peripheral blood, regardless of age. Compared to 12 week old control mice, $Lyn^{-/-}$ mice with mild disease had a small increase in CD98 neutrophils in the periphery. Importantly, similar to human disease, $Lyn^{-/-}$ mice exhibiting moderate to severe disease had a markedly increased number of CD98 neutrophils in their blood and spleen compared to $Lyn^{-/-}$ mice with mild disease. This indicates that CD98 neutrophils are behaving similarly in the $Lyn^{-/-}$ mouse model to human SLE patients. We believe the Lyn-/- mice therefore represent a useful model to understand how the CD98 neutrophil subset contributes to SLE.

Ongoing research

We have strong evidence to show that CD98 neutrophils are pathogenic and may contribute to inflammation and tissue damage in SLE. We also have *in vitro* data to show that targeting CD98 may limit their pathogenic capacity. To evaluate this directly, we will specifically delete CD98 from all granulocytes and granulocyte progenitors by crossing the $CD98^{flox}$ mice with the

neutrophil conditional MRP8^{Cre} mice. Using funds provided by Arthritis Australia**,** we have purchased CD98^{flox} mice from Jackson laboratories and established a colony at WEHI. MRP8^{Cre} mice are available in-house at WEHI and we have commenced breeding a CD98 conditional neutrophil knockout mouse. Ongoing research continuing in 2023 will generate Lyn$^{-/-}$ mice lacking CD98 in this neutrophil subset to determine whether targeting CD98 can limit neutrophil-mediated inflammation and tissue damage and thereby decrease the severity of SLE.

Grant outputs

Publications

Martin KR, Day JA, Hansen JA, D'Silva DB, Wong HL, Garnham A, Sandow JJ, Nijagal B, Wilson N, Wicks IP**.** (2023) *CD98 defines a metabolically flexible, proinflammatory subset of low-density neutrophils in systemic lupus erythematosus.* Clin Transl Med. 2023 Jan:13(1)

Conference presentations

- Oral presentation, Society for Leukocyte Biology Annual Meeting 2022 (Hawaii, USA). CD98 defines a metabolically flexible, proinflammatory subset of low-density neutrophils.
- Oral presentation, Australian Inflammation Centers 2022 Symposium (Melbourne). CD98 – a novel regulator of inflammation in rheumatoid arthritis
- Oral presentation, Australian Rheumatology Association (ARA) Annual Scientific Meeting 2021 (Virtual). Identification of a novel pathogenic neutrophil subset in SLE.

Plain language summary

What is the problem?

Systemic lupus erythematosus (lupus) is an autoimmune disease where the body begins to attack itself. People with lupus develop severe inflammation and tissue damage that affects many different organs and tissues throughout the body including the skin, joints, heart, kidney, nerves, and blood vessels. Over 20,000 Australians and 5 million people worldwide are estimated to be living with this disease. Despite recent advances in our understanding of what causes lupus and some improvement to available treatments, the prognosis for many patients remains poor and quality of life is severely impacted. Treatments for this disease can be ineffective, non-specific, have many serious side effects and can leave patients susceptible to life threatening infections. In fact, only two new drugs have been approved for lupus in the past 20 years and these new treatments unfortunately have limited use in the clinic as only a very small group of patients benefit from their use. Therefore, there is a need to identify new causes of this disease so we can develop new and better therapies to treat lupus.

Identification of a new population of disease-causing immune cells in lupus

One type of white blood cells thought to contribute to the inflammation and organ damage in lupus are neutrophils. Neutrophils are the most abundant immune cells in the blood and play an essential role in protecting the body against infection. On the other hand, hyperactive neutrophils can cause chronic inflammation and tissue damage in a range of autoimmune diseases. Indeed, recent research in both human patients and animal models of lupus have shown that neutrophils can release toxic molecules and signals that cause inflammation, organ, and tissue damage, all of which are associated with lupus. Importantly, while neutrophils were long considered to be a homogenous population of cells, there is compelling evidence that certain subsets of neutrophils are disease causing and can promote inflammation and tissue damage in human disease but are absent in healthy people.

Using advanced technologies, we were the first to identify a new neutrophil population that is present in the blood of lupus patients with active disease. These neutrophils can be identified by a protein on their cell surface called CD98. CD98 can transport a type of fuel called amino acids into cells, and these amino acids are then used by cells to generate energy.

Our idea

We propose that CD98 neutrophils cause inflammation and tissue damage in lupus and that targeting these disease-causing neutrophils could be a new way to treat this debilitating autoimmune disease. We will address our aims using blood collected from human lupus patient as well as from mouse models of lupus.

What did we discover?

Biomarkers are something that doctors can assess in patients that tells them how severe disease is, whether treatments are working, whether patients are in remission or are having a flare of symptoms. Biomarkers in lupus are severely lacking and this hinders the way doctors can properly manage these patients and impedes development of new therapeutics. Using funding from Arthritis Australia, we collected blood from human lupus patients and showed that CD98 neutrophil levels correlated with "disease activity" (Martin et al, 2023, Clinical and Translational Medicine). This means that patients with severe lupus symptoms had more of these neutrophils in the blood when compared to patients with mild disease and this population was completely undetectable in healthy people. This exciting finding suggests that the levels CD98 neutrophils could represent a "biomarker" to be used by doctors as a simple and effective test to help guide patient care or to assess whether treatments are working. In addition to showing that CD98 neutrophils correlate with the severity of lupus, we also show that they can cause an inflammatory response in patients. For example, we showed that CD98 neutrophils produce proinflammatory signals, release toxic molecules that cause cellular and tissue damage and are more metabolically active which enhances their ability to cause damage.

To understand exactly how CD98 neutrophils contribute to lupus and to determine if targeting this population could be new way to treat this autoimmune disease, we turned to a mouse model that develops many of the same clinical features as human lupus. These mice develop systemic inflammation, produce of self-targeting proteins called autoantibodies and show damage to organs such as the kidney. These mice develop mild lupus symptoms at 24 weeks of age and show severe signs of disease at 36 weeks of age. Given that in human disease, CD98 neutrophils are found in patients with lupus but not in healthy people, we asked the

question of whether these cells were also found in the mouse model of lupus. We looked at mice with no disease symptoms (12 weeks of age), mild (24 weeks of age) or severe symptoms (36 weeks of age) and compared them to normal mice. In these experiments, we found that before disease developed (12 weeks), the lupus mice had equivalent levels of CD98 neutrophils as normal healthy mice. Similar to human patients, lupus mice with mild or severe disease symptoms had a large increase in the number of CD98 neutrophils in their blood compared to normal healthy mice. This indicated that CD98 neutrophils are behaving similarly in the lupus mouse model as they are in human patients and means we can use these mice better understand the role these neutrophils playing in disease and find ways to target them and limit their disease causing capacity.

Where to next?

We have strong evidence to show that CD98 neutrophils contribute to lupus. We also have exciting data to show that if we target CD98 on this neutrophil population in a test tube we can limit the amount of damage they can cause. The next step of this project is to determine if targeting CD98 on the disease causing neutrophil population can prevent or treat lupus. To do this, we have used funds from Arthritis Australia to purchase genetically modified mice that do not have CD98 on the surface of the neutrophils. Our next step is to breed these mice with the lupus mice and determine whether absence of CD98 can limit the damage these neutrophils can cause and decrease the severity of lupus symptoms.

How will this benefit people with lupus?

Biomarkers represent an essential part of clinical practice and are used in almost every step of patient care. Biomarkers that predict disease outcomes, remission, and long-term adverse events in lupus are severely lacking and this hinders the way doctors can properly manage these patients and impedes development of new therapeutics. We have developed an easy and simply test clinicians can use to guide the way they treat patients. Furthermore, we are providing the proof-of-concept evidence that CD98 on these disease causing neutrophils represents an exciting new therapeutic target to treat lupus.