LOW DENSITY GRANULOCYTES (LDG) IN THE PATHOGENESIS OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE): TARGETS FOR THERAPY?

Source picture: https://www.huidziekten.nl/zakboek/dermatosen/mxt/malar-rash.htm, Malar rash (Butterfly erythema)
Summary

Recently, it has become more apparent that Low Density Granulocytes (LDG) are a subgroup of neutrophils with possible involvement in different auto-immune diseases. LDGs have increased Neutrophil Extracellular Trap (NET) formation activity, increased type I Interferon (IFN) production, lower apoptosis rates and, pro-inflammatory effects. LDGs possess cell-surface molecules which are characteristic for an activated phenotype, yet the morphological appearance of their nuclei is more like that of an immature neutrophil. This difference supports the contention of LDGs being a subgroup of neutrophils.

The increased pro-inflammatory activity of LDGs may have detrimental effects on the development and progression of Systemic Lupus Erythematosus (SLE). SLE is an auto-immune disease characterised by chronic, widespread inflammation. This inflammation and autoimmunity affects multiple organs and have different manifestations such as glomerulonephritis, atherosclerosis and skin rash. Recent studies have shown that patients with SLE have a higher cell-count of LDGs compared to healthy patients and that a higher LDGs cell-count correlated with more double-stranded-DNA antibodies. These antibodies are known to be present in SLE patients. This together promotes the thought of LDGs being a key-player in the development and progression of SLE. Considering the current therapeutic approach, namely alleviating symptoms of the disease, more specific therapeutic targets are needed in order to minimize the side-effects and to preserve the defensive capabilities of the immune system.

The purpose of this thesis is to obtain more insights in the activity of LDGs in SLE and to provide possible therapeutic targets for LDGs to improve the treatment of patients with SLE. Intervention in the activity of LDGs seems promising and could provide therapeutic possibilities in the future. Since LDGs have a pro-inflammatory phenotype, production of factors such as type I IFNs are increased. Literature has shown that inhibition of type I IFNs reduces SLE activity, which suggests inhibiting LDG may indirectly reduce type I IFNs as well. Furthermore, inhibition of NET formation also reduced manifestations of SLE such as glomerulonephritis. Additionally, neutrophils are thought to be involved in the pathogenesis of SLE as well, since depletion of neutrophils showed a reduced SLE phenotype. For this reason, depletion of LDGs may reduce SLE activity to a greater extent since these cells have an increased pro-inflammatory phenotype compared to normal neutrophils. However, current literature still remains controversial on the effect of inhibition of LDGs activity, implying the value of more knowledge on this topic.
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Introduction

1.1 Pathogenesis of SLE

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterised by chronic, widespread inflammation. This inflammation is the result of depositions of immune complexes composed of self-antigens and antibodies against these self-antigens in vessel walls of multiple organs. Manifestations of the disease can occur in multiple organs and may include nephritis, atherosclerosis, skin rash and vasculitis. The pathogenesis of SLE is not fully understood and it is thought that multiple factors contribute to the development of the disease. Genetics, epigenetics and environmental factors such as smoking, UV light and vitamin D deficiency are some of the main contributors involved in the pathogenesis. Furthermore, oestrogens seem to be an important factor involved in the pathogenesis as well, since SLE mainly affects young women. All these factors that contribute to the development and progression of SLE reflects the complexity of the disease.

![Figure 1 Clinical manifestations of SLE. Source: Crampton et al., 2014, Multi organ involvement in SLE, article: "Linking susceptibility genes and pathogenesis mechanisms using mouse models of SLE"](image)

Since SLE is an autoimmune disease, tolerance to self-antigens is lost and auto-antibodies are formed. In SLE it is known that Anti-Nuclear-Antibodies (ANA) and antibodies that react to double stranded DNA are present. This reflects the systemic nature of the disease since these antigens are widely present in the body. Because formation of auto-antibodies is a prominent characteristic of SLE, it is assumed that B cells and T cells play a major role in the disease pathogenesis. T cells are highly active in SLE and stimulate B cells to produce antibodies. In the case of an auto-reactive B cell, excessive amounts of auto-reactive antibodies end up circulating and, upon encounter with the auto-antigen, form complexes that deposit in vessel walls inducing inflammation. The induction of autoimmunity is not fully understood. There are however several factors that have been identified that are thought to play a role in the process. For example, it has been postulated that molecular mimicry by pathogens can play an important role in this loss of self-tolerance. This was shown in a study on SLE pathogenesis, in which it was observed that many SLE patients had auto-antibodies against the antigen Ro. Ro is a polypeptide which is present in the nucleus and cytoplasm and are detected earliest in SLE patients. Interestingly, animals immunized with either Ro or Epstein-Barr virus nuclear antigen-1 (EBNA-1) developed symptoms comparable to SLE. This together supports the contention that viral antigens are the initial trigger of disease development and that molecular mimicry plays a role in the loss of tolerance in SLE. However, molecular mimicry and therefore induction of SLE may also derive from intrinsic pathological processes as will be discussed later.

1.2 Current treatment

Current treatment of SLE consists of non-specific targeting of inflammation with glucocorticoids, anti-malarial therapy, mycophenolate, cyclophosphamide and methotrexate. Unfortunately, some of these therapies are highly toxic which may induce manifestations similar to SLE. This essentially shifts the problem from SLE associated pathologies to medicine associated complications, ultimately resulting in similar morbidity. The current therapies are mostly targeted towards common manifestations seen in SLE patients, most particularly the ongoing inflammation in several organs. This entails that current therapies are focussed on alleviating symptoms and are therefore not a curative therapy for SLE.

Better fitting treatment may be achieved by the use of immune modulatory biologicals; antibodies that interfere in different immunological processes by means of depleting pro-inflammatory factors or inhibiting receptors to downregulate the activity resulting from these factors or receptors. Various biologicals targeting different immune cells or immune factors are known to be beneficial in various
immune mediated diseases such as Rheumatoid Arthritis.

Rituximab has already been proven to be effective in treating SLE patients, with remission of 67% 1 year after administration. Rituximab is a biological that targets CD20, which is a cell-surface molecule found on B cells. Therefore, administering Rituximab results in B-cell depletion in subjects. Belimumab is also used in treating SLE and is a biological against B-cell Activating Factor/B-Lymphocyte stimulator (BAFF/BLys). BAFF/BLys are survival factors for B cells and induce differentiation towards plasma cells. Patients with SLE are known to have increased levels of BLys, which leads to the contention that it is an excellent target for therapy. However, controversial results have been published on the targeting of B-cells, which will be discussed later on.

1.3 Need for a more specifically targeted intervention

As mentioned before, progression of SLE will continue under the current symptomatic treatment and this treatment may have unwanted side effects since its targets are rather aspecific. Nevertheless, the survival rate of SLE is now 90% over the first 10 years after diagnosis, which demonstrates that a significant improvement has been made compared to 60 years ago, when the survival rate was just 50% over the first 5 years after diagnosis. This is mostly due to improved treatment of complications.

However, even with this improvement, quality of life can still be gained by obtaining more knowledge on the pathogenesis of SLE and using this to design an improved therapy. With more targeted therapy, the patient will suffer less treatment related toxicity and progression of the disease could even be halted altogether instead of solely alleviating the symptoms.

Considering the involvement of B-cells in the pathogenesis of SLE, targeting B-cells seems to be a suitable therapy. However, this is not always successful which is illustrated by a study on inhibition of B-Cell maturation antigen (BCMA). BCMA is the receptor for BAFF on B-cells and therefore important for the survival and differentiation. Considering the contribution of B-cells to disease activity in SLE, a less severe SLE phenotype would be expected once BCMA is inhibited. However, BCMA inhibition showed a rather controversial effect, as a more severe lupus phenotype was observed.

Furthermore, since B cells are important in the adaptive immune response, depletion with Rituximab or Belimumab results in complications such as bacterial or viral infections.

![Figure 2: Mechanisms thought to be involved in the development of loss of tolerance in SLE. Plasmacytoid dendritic cells exposed to self-antigens can induce a cascade of events inducing auto-reactive B cells. Self-antigens may be derived from NETs or apoptotic cells which are not cleared effectively. Recognized self-antigens will be presented to T-cells after which these cells activates B-cells to differentiate into plasma cells and auto-antibodies will be produced specific against this self-antigen. Source: Tsokos et al., 2016, article: “New insights into the immunopathogenesis of systemic lupus erythematosus”](image-url)
Altogether, the controversy regarding B-cell targeting and the broad targeting of current therapies such as corticoids suggests the need for a more targeted therapy of different key-players in the pathogenesis of SLE.

Since SLE is an auto-immune disease characterised by a major involvement of the immune system and it’s factors\textsuperscript{1}, biologics may provide very promising therapy by targeting factors that are more specific to SLE. For this reason, several potential targets will be discussed later on.

1.4 Recent discoveries

Involvement of the innate immune system is plausible and therefore a potential target for therapy. More specifically, neutrophils, may be a key player in the pathogenesis of SLE\textsuperscript{11}. Neutrophils are essential in the defence against microbial pathogens. They are the most abundant white blood cells in the body and have several defence mechanisms such as pro-inflammatory cytokine production, phagocytosis and Neutrophil Extracellular Trap (NET) formation\textsuperscript{11}. NET formation is a mechanism in which a neutrophil releases chromatin from the nucleus in order to trap microbes\textsuperscript{11}. Once NETs are released, cell-lysis will occur which is termed NETosis. Normally, NETs will be cleared after NETosis but in SLE this happens less efficiently, resulting in the exposure of self-antigens which are normally not accessible\textsuperscript{17}. This open access to antigens which are supposed to be stored in the cell, is hypothesised to contribute to loss of tolerance\textsuperscript{17}.

Recently, it has been shown that in different auto-immune diseases and especially SLE, there is a subgroup of neutrophils present which have an activated phenotype and differ in appearance compared to normal neutrophils\textsuperscript{15}. This subgroup does not have a granular appearance, which is why they are called Low Density Granulocytes (LDG)\textsuperscript{16}. In SLE, 50\% of the peripheral blood nuclear cells are LDGs. This abundance shows that if LDGs are actually involved in the pathogenesis, targeting these cells or interfering in cell activity could have significant effects on manifestations of SLE.

The appearance of LDGs are rather unusual. While LDGs express surface markers that are similar to activated, mature neutrophils, their morphological appearance is more like immature neutrophils\textsuperscript{18}. Furthermore, cell-surface markers for immature neutrophils are absent\textsuperscript{13}. The activated phenotype is supported by the increased expression of cytokines such as Type I Interferons (IFN), lower apoptosis rates and a higher capacity to produce NETs\textsuperscript{13}. Since increased numbers of LDGs are correlated with higher disease activity in SLE\textsuperscript{19}, the contention arises that they are a contributor to the pathogenesis of SLE.

1.5 Aim of thesis

In this thesis, I will review and discuss the current knowledge on the role of LDGs in the pathogenesis of SLE. First, I will provide a short overview of neutrophil and LDGs development. Next, I will review the current evidence implicating LDGs as pathogenic players in SLE and discuss whether interfering with the activity of LDGs could be a potential novel therapeutic approach for SLE.

Involvement of Low Density Granulocytes in the pathogenesis of SLE

2.1 Development of neutrophils and LDGs

Neutrophils are derived from the bone marrow and undergo different phases of maturation\textsuperscript{20}. The precursor cell for all white blood is the pluripotent hematopoietic stem cell. Haematopoiesis is the formation of blood cells. The pluripotent hematopoietic stem cell can differentiate into several cells such as red blood cells, granulocytes and macrophages in which the neutrophil belongs to the granulocytes. Different stages are observed during their development. To begin, a neutrophil starts as an immature cell which lacks a granular appearance and

![Stages of neutrophil development](http://eclippath.com/atlas/immature-pmn-comp-2/)

**Figure 3: Developmental stages of neutrophils.** The development starts with a mononuclear myelocyte which has a more rounded nucleus. The metamyelocyte is the next stage which is followed by the band-cell. Here, the nucleus already starts to obtain a more lobular structure. The last stage is the mature neutrophil which has a half a granular, multi-lobular appearance. Source: [http://eclippath.com/atlas/immature-pmn-comp-2/](http://eclippath.com/atlas/immature-pmn-comp-2/), stages of neutrophil maturity.
has a more rounded nucleus, called a myelocyte (Figure 3). Further developmental stages include the metamyelocyte, the band-cell and the mature neutrophil. Once the neutrophil matures, the nucleus becomes multi-lobular and the neutrophil obtains a more granular appearance. Interestingly, LDGs are most comparable to the immature band cells (Figure 4)\textsuperscript{18}.

A key factor involved in the development and maturation of neutrophils is Granulocyte-Colony Stimulating Factor (G-CSF)\textsuperscript{18}. This factor is also important for survival of mature neutrophils and migration from the bone marrow to the periphery\textsuperscript{21}. Therefore, G-CSF may be one of the factors responsible for the difference in developmental stage appearance of LDGs and neutrophils. This suggests that targeting G-CSF may lead to new therapeutic possibilities. The potential of an intervention in this development will be discussed later.

![Figure 4: The morphological appearance and difference between neutrophils and LDG. Left: neutrophil with a multi-lobular appearance. Right: an LDG with an appearance comparable to a band-cell. Source: Fu et al., 2014, Cytospins of PBMCs, article: Neutrophil-like low-density granulocytes are elevated in patients with moderate to severe persistent asthma.](image)

### 2.2 LDG characteristics

In the majority of SLE patients, LDGs can be detected in the circulation. Although LDGs may also be present in healthy subjects, the prevalence is much lower compared to SLE patients\textsuperscript{22}. In addition, another study showed that higher numbers of LDGs were correlated with higher levels of anti-dsDNA antibodies\textsuperscript{21}. These two studies strongly indicate the involvement of LDGs in SLE pathogenesis and also suggest that LDGs could be directly related to increased disease activity. Moreover, the pro-inflammatory effect of LDGs might even be the main cause of exacerbations.

It is known that LDGs produce higher amounts of type I IFN\textsuperscript{15}. A pro-inflammatory phenotype therefore seems plausible as, type I IFN are known to be correlated with inflammation\textsuperscript{24}. Studies investigating the effects of LDGs supernatants on T cells appear to confirm this contention since this resulted in induction of pro-inflammatory cytokine production by the T cells\textsuperscript{25}. Interestingly, LDGs also elicited an increased cytotoxic effect on endothelial cells after overnight exposure compared to normal SLE neutrophils (Figure 5)\textsuperscript{26}.

Normally, neutrophils differ in morphological appearance depending on their developmental stage\textsuperscript{11}. Immature neutrophils have a more rounded nucleus and mature neutrophils’ nuclei appear multi-lobular. As mentioned earlier, LDGs have a contradicting profile. Their nuclei appear more monocyte-like and thus more immature\textsuperscript{18,27} yet, based on the expression of cell-surface molecules, LDGs resemble activated, mature neutrophils.

![Figure 5: Increased cytotoxic effect of SLE LDG compared to normal SLE neutrophils. Endothelial cells showed an increased percentage of apoptosis after overnight exposure to lupus neutrophils and LDG. This induction of apoptosis was even greater in SLE LDG compared to SLE neutrophils. Source: Denny et al., 2019, article: A distinct subset of proinflammatory neutrophils isolated from patients with Systemic Lupus Erythematosus induces vascular damage and synthesizes type I IFNs.](image)

Once neutrophils are activated during inflammation, their production of cytokines is increased, their expression of cell-surface molecules like CD11b and CD66bis upregulated\textsuperscript{29} and their apoptosis rates are lowered\textsuperscript{30}. CD11b is an integrin\textsuperscript{31} and CD66b is a GPI-anchored glycoprotein of the Carcinoembryonic Antigen Family\textsuperscript{32}. Both are activation markers for granulocytes. Since these activation-related factors are also present on LDGs, these cells are considered to be in an activated state.

Another cell-surface molecule of interest is CD15. The expression of CD15 was observed to be significantly increased on LDGs compared to neutrophils (Figure 6)\textsuperscript{18}. However, it is important to mention that this was also observed in LDGs obtained from healthy controls, suggesting that this is a general feature of LDGs that is not related to increased disease severity in SLE patients. Nevertheless, the level of CD15 expression could be helpful in distinguishing LDGs from normal neutrophils.
Interestingly, it was observed that the expression of CD11b and CD66b was not upregulated in relation to SLE activity. However, the number of LDGs was increased\(^2\). This suggests increased release of LDGs during active disease instead of solely upregulation of solely upregulation of these cell-surface markers. The fact that higher LDGs cell-counts have been detected in SLE patients with active disease as well seems to corroborate this contention\(^3\).

2.3 Activated phenotype of LDGs

The activated phenotype of LDGs is characterized by having higher production of type I IFN, more NET production and lower apoptosis rates\(^{15,28}\). These factors may therefore be involved in the pathogenesis of SLE as well.

NET formation is a complex process with involvement of multiple factors. Until recently, not much was known about this phenomenon\(^16\). Research has now shown that the formation of NETs is dependent on the production of Reactive Oxygen Species (ROS), which is produced by the NADPH oxidase complex (NOx)\(^17\). Furthermore, PAD4 is also known to be an essential enzyme in the formation of NETs\(^11\).

The increased NET formation of LDGs is supported by the fact that SLE patients have more detectable cell-free DNA (cf-DNA)\(^{40}\). As mentioned before, a higher LDG cell-count is often seen in combination with more ds-DNA-antibodies. Since LDGs have more NET formation and may thus provide more ds-DNA antigens, the notion arises that NETs may induce loss of tolerance.

NETs can also induce cytokine production and promote inflammation via the activation of the NLPR3 inflammasome\(^15\). This induces the production of IFN\(\alpha\), IL1\(\beta\) and IL18, which are pro-inflammatory cytokines. This further supports the contention that the presence of NETs promotes the inflammatory response seen in SLE and are therefore involved in the pathogenesis of SLE.

An important cause of mortality in SLE is atherosclerosis\(^33\). Interestingly, NETs seem to be involved in this manifestation. In a study on High Density Lipoprotein (HDL) alteration by NETs, it was observed that NETs induced HDL to become less capable of transporting cholesterol and had an activating effect on macrophages\(^44\). Therefore, NETs may promote the formation of atherosclerosis. This again supports the involvement of NETs in the pathogenesis of SLE.

Besides their contribution to SLE pathogenesis, NETs are also thought to be involved in triggering the onset of SLE\(^39\). More specifically, the chromatin which is released during NETosis could provide of ds-DNA antigens which may results in the formation of auto-antibodies. For this reason, NETs may be an intriguing target for therapy in SLE. Since NETs are associated with increased levels of anti-ds-DNA and LDGs show increased production of NETs, targeting LDGs is even more hopeful. This is also supported by the aforementioned increased number of LDG associated with SLE activity.

As mentioned before, LDGs produce more type I IFN, which makes type I IFN an important cytokine\(^15,26\). It is known that IFN can promote survival in B cells due to BAFF/BLys and APRIL stimulation. These are survival factors of B cells\(^14\). This suggests that type I IFN production of LDGs could also contribute to the loss of tolerance of B cells due to over-activation.

Collectively, increased type I IFN production and NET formation by LDGs suggest a role for LDGs in the onset of SLE when tolerance is lost, and auto-immunity develops.

2.4 Involvement of LDGs in other auto-immune diseases

As more research is done on the activity of LDGs and their presence in auto-immune diseases, it becomes more evident that LDGs may be involved in the pathogenesis of different auto-immune diseases as well. Psoriasis, Rheumatoid Arthritis, Asthma and acute rheumatic fever are a few of the diseases with proven involvement of LDGs\(^16,18,27\). This makes research on LDGs timely and important since therapies aimed to inhibit the detrimental effects of LDGs could constitute a novel therapeutic approach for other auto-immune diseases as well.
Discussion

3.1 LDGs are a subgroup of neutrophils

Considering the activity and phenotype of LDGs described in the literature and discussed in this review, it is very likely that LDGs are a subgroup of neutrophils with a fundamentally different behaviour. This is based on the fact that their cell-count correlates to disease activity of SLE\textsuperscript{19}, their appearance is different than normal neutrophils and their activated phenotype\textsuperscript{26}. Inhibiting the factors responsible for their activated phenotype results in reduced SLE activity, making it highly plausible that LDGs contribute to the pathogenesis of SLE.

3.2 Intervention in development of LDGs

Several factors are involved in the development of neutrophils. As mentioned before, one of the essential factors in the development of neutrophils and in their migration from the bone-marrow, is Granulocyte-Colony Stimulating Factor (G-CSF)\textsuperscript{20}. After development, neutrophils have a short lifespan of 24 hours. However, once infection occurs, their survival will be prolonged in order to facilitate an effective immune response against the invading pathogen. Interestingly, G-CSF is also involved in this process and is thus capable of inducing delayed apoptosis in neutrophils\textsuperscript{21}. Since LDGs may have a similar development compared to that of neutrophils and have lower apoptosis rates, it is plausible that these cells are more sensitive to G-CSF. This may be due to increased receptor expression, or locally increased levels of G-CSF. This then leads to a higher number of LDGs with a lower apoptosis rates. Both of these effects ultimately result in increased LDG activity potential and could therefore lead to increased SLE disease activity. For this reason, an intervention based on G-CSF inhibition could be promising. This could be done by using biologicals which inhibit G-CSF and therefore the development of LDGs. Unfortunately, G-CSF is essential for normal neutrophil development as well. Therefore, inhibition of G-CSF may also result in inhibition of development of neutrophils and their vital role in the innate immune system. Assuming LDGs are more sensitive to G-CSF, using G-CSF inhibition as a therapy may work once the correct dose is found. An optimal effect to complication ratio may be achieved once research with focus on dose administration is performed.

Studies have already been done on neutrophil depletion. Less glomerulonephritis was observed in neutrophil depleted mice with a genetically induced SLE phenotype\textsuperscript{15}. Depletion of LDGs may be an effective way to treat manifestations in SLE as well, since LDGs show even more pro-inflammatory activity than neutrophils in SLE. By only inhibiting LDGs, the positive defence capability of neutrophils will be preserved. It has already been shown that medium without LDGs, which was added to endothelial cells, induced a significant decrease in cytotoxicity\textsuperscript{26}. This purification of LDGs was done by labelling with markers against a subset of cell-surface molecules. Specific in vivo depletion of LDGs is challenging, since a single specific marker for LDGs has not yet been found. Nevertheless, since isolation of LDGs is already possible, it would be interesting to perform a study in which LDGs are isolated and a gene analysis is performed. This may lead to specific markers for LDGs. Once specific markers are found, biologicals targeting these markers in order to deplete LDGs can be developed.

The fact that LDGs differ in appearance compared to neutrophils but remain a subgroup of the granulocytes, suggests that the development of LDGs is different than neutrophils. If more was known about the development of LDGs, an intervention in this specific onset of different development may be of interest. A study utilizing isolated, premature LDGs and neutrophils could be performed in which their respective development is analysed to determine the difference in development between neutrophils and LDGs. If this is dependent on different specific factors, inhibition of LDG specific factors may provide a downregulation of LDG development.

It is however important to consider that LDGs may also be involved in non-pathological processes. Depletion of LDGs or inhibiting their development will therefore also affect these processes and may cause considerable complications. Not much is known about the normal physiological role of LDGs, making it an ideal topic for research.

3.3 Intervention in activity of LDGs

Research has shown that type I IFN production seems to be increased by LDG activity and that type I IFN are correlated with SLE disease activity. Therefore, targeting these cytokines directly or through inhibition of LDGs may be promising. Not only do type I IFN seem to be involved in the progression of SLE, but increased IFN production could also lead to adaptive immune system activation\textsuperscript{26}. This suggests that IFN may be involved in the severity of auto-immune diseases since production of antibodies is increased in such diseases. Additionally, it was observed that type I IFN in combination with G-CSF could induce recruitment of LDGs from the bone marrow\textsuperscript{36}. This
further suggests the importance of these factors and their potential to target them with regards to developing a therapy to slow down or diminish the progression and onset of SLE.

Several studies have shown that inhibition of type I IFN are able to decrease SLE development in mice. For example, SLE mice treated with soluble IFN-γ receptor developed less glomerulonephritis, a severe manifestation in SLE35. Inhibition of another type I IFN, IFNα, also showed improvement of the disease36. In a different study on IFNα inhibition, less expression of BAFF, TNFα, IL10 and IL1β was observed36. This is promising since it is known that factors such as TNFα, IL10 and IL1β are increased in patients with SLE (Figure 7)32. Decreased levels of BAFF could provide less activity of auto-reactive B-cells and therefore less disease activity3. Increased production of different factors and the increased recruitment of LDGs by type I IFN, underscores the importance of the type I IFN pathway in the pathogenesis of SLE.

Figure 7: Increased production of cytokines by PMA stimulated SLE LDGs. Source: Denny et al., 2019. LDG secrete increased levels of pro-inflammatory cytokines, article: A distinct subset of proinflammatory neutrophils isolated from patients with Systemic Lupus Erythematosus induces vascular damage and synthesizes type I IFNs

Type I IFN are also thought to be increased due to NET formation and as stated earlier, NET formation is upregulated in LDGs41. Therefore, targeting LDGs will decrease type I IFN not only directly but also indirectly through decreased NET formation. Furthermore, NETs may also contribute to the autoimmune reaction and disease activity directly31. Since NET formation depends on several events and substrates, targeting these factors may decrease activity of SLE.

Interestingly, patients with Chronic Granulomatous Disease (CGD) show deficiencies of NOx and are therefore unable to form NETs17. This suggests that inhibiting NOx could downregulate the NET formation which occurs in SLE. However, neutrophils from patients with CGD treated with singlet oxygen were able to form NETs again40. This suggests the importance of singlet oxygen in NET formation, making it an interesting therapeutic target. Furthermore, singlet oxygen is also related to concentration of Uric Acid, which is a metabolic breakdown product of amino acids41. This suggests that increased levels of Uric Acid could increase singlet oxygen concentration and therefore NET formation. Considering that an increased Uric Acid concentration is also present in SLE patients, which needs to be determined in advance, Uric Acid may be a suitable marker to detect disease activity at an earlier stage. If at an early stage elevated levels of Uric Acid are indeed observed, therapeutic agents could be used in order to lower the Uric Acid concentration. This may provide less NET formation and therefore less detrimental behaviour of LDGs. However, NET inhibition has contradictory outcomes in the literature. For example, NOx inhibition showed a worse SLE phenotype in mice42. Meanwhile, inhibition of an essential factor in NET formation, PAD4, showed improvement of renal, vascular and skin disease in SLE in lupus prone mouse models43.

The ROS dependency of NET formation suggests that inhibiting ROS with antioxidants could be promising. Next to the possible attenuation of NET formation this may provide, it could also provide added positive effects on tissue damage and vascular inflammation since ROS have direct cytotoxic effects. In mouse studies, antioxidants have shown impressive effects on manifestations of SLE including diminished autoantibody production, less renal insufficiency and, decreased mortality44.

The contradictory outcomes in the literature regarding inhibition of NETosis in SLE reflects the complexity of NET formation as well as involvement in SLE pathogenesis. Thus, more research is needed in order to determine the exact role of NETs in SLE development and their potential as targets for future therapy.

3.4 Future perspectives of targeting LDGs

The involvement of NET formation by LDGs might be an interesting target, as this does not only affect the progression of the disease but may be involved in the disease activity of SLE due to increased self-antigen exposure and therefore antibody deposition. However, as stated before the literature on the effect of inhibition of factors involved in NETosis is rather controversial. More
Insight in NET formation by LDGs is needed in order to find new targets that can be acted upon by medication. For example, more studies regarding inhibition PAD4 and inhibition of ROS with a focus on LDG activity could be performed. Another option would be studying NOx in more detail. Since inhibition of NOx showed a worse phenotype in SLE mice, it would be interesting to do the opposite and upregulate NOx. This may cause more NET formation since this is dependent on NOx, but still induce a reduced SLE phenotype as well. Assuming that this study provides a positive outcome, it would then be intriguing to observe a group of mice where NOx is upregulated, and ROS is inhibited. This would create a situation in which the potential positive effects of NOx upregulation and NET inhibition are combined to reap the benefits of both potential therapies.

As mentioned before, type I IFN production is significantly increased by LDGs. Clinical trials on inhibition of type I IFN using the biologicals Anifrolumab, Sifalimumab and Rontalizumab have already proven to be effective in reducing the SLE phenotype. Since LDGs may be the main source of type I IFN in SLE patients and LDGs are also known to have increased NETosis rates, targeting LDGs may provide a single therapy which targets multiple factors involved in the pathogenesis of SLE. This would result in a better, more efficient and patient friendly therapy.

Additionally, an interesting study to perform would be to measure levels of ROS and Uric Acid in SLE patients, to determine their involvement in NET formation and therefore pathogenesis of SLE. Eventually, such factors could be used as biomarkers for disease activity or may be targeted in order to reduce NET formation.

Furthermore, research on the phenotype of LDGs is needed in order to determine a specific marker for LDGs. As mentioned before, finding an LDG specific marker and developing a biological targeting this marker may provide an effective therapy.

**Conclusion**

In conclusion, LDGs seem to be a genuine subgroup of neutrophils in SLE. These cells are characterized by an activated pro-inflammatory phenotype which could have detrimental effects on the development and progression of SLE. Intervention in the activity of LDGs seems promising and could provide potential targets for therapy in the future. An important consideration to take into account is that SLE is a very complex disease and the ideal therapy should be as specific as possible in order to minimize effects on the defence mechanism of the immune system. By all means, I believe that targeting LDGs is an excellent strategy to treat SLE considering their activated phenotype. Moreover, inhibition of different factors upregulated in LDG activity showed decreased SLE activity. Targeting these factors at their source, namely LDGs, would provide a single therapeutic target. In my opinion, finding a specific marker for LDGs may be the most promising as biologicals targeting this specific marker could then be developed. Given the complexity of SLE pathogenesis, more research is needed to acquire a better understanding of the contribution of the numerous immune cells involved and how these cells interact with each other.
**Literature Research**

**LDG**

In Smartcat and PubMed I searched for *Low Density Granulocytes* to obtain more knowledge about these cells. MeSH terms I searched for were: “Low Density Granulocytes”/”LDG”/subgroup neutrophils/low buoyant granulocytes.

In order to obtain more knowledge about the pathophysiology of SLE, I used MeSH terms: SLE/Systemic Lupus Erythematosus/Pathophysiology SLE/Pathophysiology Systemic Lupus Erythematosus/Pathogenesis SLE/Pathogenesis Systemic Lupus Erythematosus.

**LDG in SLE**

Furthermore, I combined these 2 search options to obtain information about the role of LDG in SLE. However, not much literature is available, which led me to delve into the involvement of normal neutrophils in the pathogenesis of SLE.

MeSH terms LDG in SLE: “LDG in SLE”/”Low Density Granulocytes in Systemic Lupus”

MeSH terms neutrophils in SLE: “neutrophils in SLE”/”neutrophils in Systemic Lupus”.

This led to literature about interventions targeting activity which is known to be increased in LDG.

**Recent discoveries**

All the literature I found regarding B and T cells I mostly filtered out since I was searching for more insights about the innate immune system involvement in SLE. Also, since I focused on potential biologicals to target different factors of LDG, I preferably filtered for results of the last 5 years since biologicals are fairly recently. Once I did this, I also searched without this filter to find more studies which fitted my interest as well. By doing this, I first discovered most recent findings, after which I also found older, but still important performed studies.

Furthermore, I organized the literature in different folders:

- Pathogenesis of SLE
- LDG in SLE
- Activity of LDG
- Therapy of SLE
- Potential therapy for SLE
- LDG in auto-immune diseases
References


27. CCDB


