Targeting autoimmunity in renal diseases: focus on neutrophil extracellular traps and autoreactive B-cells

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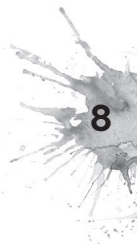
Summary and discussion

SUMMARY

ANCA-associated vasculitis (AAV) and systemic lupus erythematosus (SLE) are severe, but rare autoimmune disorders, that commonly affect the kidneys and lead to increased morbidity and mortality in patients. There is accumulating evidence on the role of excessive NET formation and impaired NET degradation in the pathogenesis of both AAV and SLE[1-7]. NETs are a source of autoantigens in both AAV and SLE that can initiate the humoral autoimmune response and can cause direct glomerular inflammation and damage. NETs have been shown to trigger autoreactive B-cells to produce disease-relevant autoantibodies[3]. Specifically in SLE patients, NETs can form immune complexes with autoantibodies that further enhance ICx-mediated inflammation[2].

The current concepts on the role of NET formation in the pathophysiology of AAV and SLE were summarized in **chapter 2**. This review provides a translational perspective on the clinical implications of NETs, such as potential approaches that could target NET formation in these renal autoimmune diseases. To study NETs accurately in health and disease, NET formation should be quantified in a specific, sensitive and objective manner. The quantification of NET formation can be performed using different techniques[8]. The properties of the techniques determine the specificity, sensitivity, objectivity, and speed to detect NET formation. In **chapter 3**, we provided a protocol to quantify *ex vivo* NET formation in a highly-sensitive, high-throughput manner by using three-dimensional immunofluorescence confocal microscopy. This protocol can be applied to quantify NETs to study them in health and disease and to evaluate potential therapeutic approaches that target NET formation.



This protocol was applied to study NET formation in a large cohort of AAV and SLE patients (**chapter 4**). In this study, we showed that both sera from AAV and SLE patients induced excessive NET formation, as compared to healthy individuals. The amount of NET formation correlated significantly with disease activity for both patient cohorts. Moreover, in this study we demonstrated that the morphology, kinetics, induction pathways and composition of NETs was intrinsically distinct in AAV patients as compared to SLE patients. This study showed lytic NET formation in AAV after hours versus rapid non-lytic NET formation coinciding with clustering of neutrophils in SLE within minutes. AAV-induced NET formation was triggered independent of (ANCA)-IgG whereas SLE-immune complexes (ICx) induced NET formation through FcγR-signaling. AAV-induced NET formation was dependent of reactive oxygen species and peptidyl-arginine-deaminases and was enriched for citrullinated histones, all in contrast to SLE-induced NETs. SLE-induced NETs had immunogenic properties including NET-bound HMGB1, enrichment for oxidized mitochondrial DNA, and were involved in ICx formation.

In SLE patients, novel B-cell targeted therapies aim to target autoantibodies and autoreactive B-cells, in contrast to non-specific conventional immunosuppressive treatments. Currently, most B-cell targeted strategies, are off-label treatments for SLE patients. Therefore, it is important to get an in-depth insight in the immunological effects of these therapies to further improve our knowledge on B-cell targeted treatments in SLE patients. To do so, we assembled sera from different SLE cohorts treated with 1) rituximab (RTX) and belimumab (BLM), 2) bortezomib (BTZ) or 3) RTX, to study the effects of these therapies on autoantibodies, immune-complex formation and NET formation (**chapter 5**). In this reverse translational study, we demonstrated that autoantibody levels decreased upon each treatment strategy. However, the extent of targeted autoantibodies was most significant for RTX+BLM, both in a quantitative manner (reduced autoantibody repertoire) as well as in a qualitative manner (reduced titers of low, medium and high-avidity anti-dsDNA autoantibodies). These effects were less pronounced for RTX only and not observed in BTZ-treated patients. Especially the reversal of anti-C1q to seronegative was associated with reduced ICx-mediated inflammation and clinical disease activity, which happened most frequent after RTX+BLM, less after RTX and not after BTZ treatment. These observations collectively demonstrate the relevance of in-depth monitoring of the immunological effects of B-cell targeted strategies that have potential implications for the clinical practice.

In AAV patients, RTX is an FDA/EMA approved therapy, which is recommended both for remission-induction and maintenance treatment[9-13]. RTX was shown to have the same efficacy as cyclophosphamide (CYC), which has been the longstanding standard of care as remission-induction treatment[9,10]. However, after RTX remission-induction therapy, AAV patients have a relatively high rate of relapses during medication-free maintenance. These observations emphasize the need for early biomarkers that can predict and thereby potentially prevent relapses. Recent studies have suggested monitoring of ANCAs and B-cells to guide maintenance treatment with RTX[11-13]. However, the predictive value of ANCAs and B-cells for relapses remains a matter of debate. To study this, we retrospectively investigated the relation of ANCA positivity and/or the return of B-cells with the occurrence of relapses in a large cohort of AAV patients that were treated with RTX (**chapter 6**). In PR3-ANCA positive patients, 96% of the relapses occurred with persistent or reappearance of PR3-ANCA positivity, often in conjunction with B-cell repopulation. Absence of PR3-ANCA positivity and B-cells after RTX was highly predictive of a relapse-free status. Although MPO-ANCA positive patients were a relatively small group, all relapses occurred with persistent MPO-ANCA positivity and B-cell repopulation. This study demonstrated that monitoring of ANCA and B-cell status could guide therapeutic decisions to prevent relapses in AAV patients after RTX as remission-induction regimen.

The re-occurrence of ANCAs, often followed by relapses, despite B-cell depletion with RTX implies that there is minimal residual autoimmunity (MRA) re-occurring in the B-cell compartment of AAV patients. We studied MRA with Euroflow-based highly sensitive flow cytometry (HSFC) and PBMC cultures in **chapter 7**. Here we demonstrated that despite significant reductions in circulating B-cell numbers after RTX, small numbers of B-cells always remained detectable when employing Euroflow-based HSFC. Residual B-cells after RTX were predominantly memory B-cells and $CD20^-$ plasma cells. Changes in ANCA levels associated predominantly with changes in circulating naive, switched or double negative (DN) memory B-cells but not with plasma cells. Within the residual B-cells present after RTX, we demonstrated the presence of ANCA-specific memory B-cells indicative of MRA in AAV patients.

DISCUSSION AND FUTURE PERSPECTIVES

The aim of this thesis was to gain more insight on the role of NETs, autoantibodies and autoreactive B-cells in the pathogenesis of both AAV and SLE. Moreover, this thesis aimed to increase our understanding of the humoral autoimmune response and to translate our knowledge to improve the targeting of autoimmunity in AAV and SLE patients and improve their clinical care.

The treatment of AAV and SLE patients is shifting from non-specific conventional immunosuppressive drugs to more targeted and individually-tailored therapies. Nevertheless, the majority of renal autoimmune disease patients are currently still treated with non-specific conventional immunosuppressive drugs, such as mycophenolate mofetil (MMF), cyclophosphamide and prednisolone, which are all associated with high rates of adverse events including infections, gonadal toxicity, malignancy, osteoporosis, diabetes, thromboembolic and cardiovascular disease[14-16]. Ideally, immunosuppressive treatment for AAV and SLE patients would encompass specific targeting of the pathogenic culprits of the disease without affecting other components of the healthy immune system or body. A reduction of autoantibodies, or even reversal to negativity, upon immunosuppressive treatment was associated with a beneficial clinical outcome in both AAV[17-20] and SLE[21-25] patients. B-cell targeted therapies precisely target autoantibodies and autoreactive B-cells and are therefore an attractive therapeutic option. During the maintenance treatment phase after patients have reached clinical remission with B-cell targeted therapies, ideally the timing and intensity of re-treatment is individually tailored based on relevant biomarkers that reflect residual autoimmunity. In the following paragraphs, we will further address the relevance of our findings and its implications for future research.

NET formation in AAV and SLE

NET formation is involved in the pathogenesis of both AAV[26,27] and SLE[1-3] and correlated with disease activity. Our study on AAV- and SLE-induced NET formation underscored that different disease-specific triggers lead to NET formation through different mechanisms, resulting in NETs with distinct compositions and immunogenic properties[7]. It has been recognized by several groups that there are many different pathways that lead to expulsion of extracellular DNA depending on the specific trigger[28-30].

The identification of specific triggers and molecular mechanisms of NET formation in AAV and SLE is relevant for future studies on potential new therapeutic targets. In this thesis, we provided a detailed protocol describing a highly-sensitive broadly applicable assay for the semi-automated quantification of *ex vivo* NET formation upon different stimuli[31]. Extracellular DNA derived from NET formation can be the result of distinct death pathways, including

NETosis, necroptosis, pyroptosis, ferroptosis, or even a non-lytic process, called vital NET formation. The advantage of avoiding NET specific markers in this assay, allows to assess all forms of NET formation leading to the extrusion of DNA by neutrophils, as complete and objective as possible, with the potential of high-throughput screening and close relation to the *in vivo* situation. This assay is not only valuable to assess NET formation in AAV and SLE patients, but can also be used to assess NET formation in other diseases.

In AAV the specific trigger(s) of NET formation are still not completely resolved. Some studies show that ANCAs induce NETs[26,27], while we and others have previously shown that NET formation was not dependent on ANCA IgG[32,33] and/or ANCA IgA[32]. Also, neither inflammatory factors such as IL-8, CRP, TNFα nor C5a were involved in NET formation[32]. Possible NET-inducing serum factors could be a combination of cytokines and/or DAMPs[34]. In addition, ANCA IgM could be a possible trigger, however it has not been studied in the setting of NET formation yet. ANCA IgM has been detected in AAV patients and was associated with disease activity[35]. In SLE patients, we and others demonstrated that SLE-specific autoantibodies (anti-RNP, -dsDNA, -C1q) form IgG immune complexes (ICx) that induce NET formation[1,2,7,36]. Specifically in SLE, this phenomenon creates an amplification loop where NET components induce autoantibodies leading to ICx, which subsequently trigger NET formation and perpetuate the phenomenon.

Several treatments have been reported to decrease *in vitro* and/or *ex vivo* NET formation in mice models. Among these are corticosteroids, which represent (still) the cornerstone treatment for both AAV and SLE patients. Interestingly, corticosteroids decreased *in vitro* (mouse, horse and human) and *in vivo* (mouse) NET formation[37-39]. Because the trigger of NET formation in AAV is not well defined, inhibition of NETs could be focussed on targeting of the potential pathways involved in AAV-specific NET formation, such as the necroptosis pathway. Necroptosis has been highlighted to be involved in AAV-induced NET formation and could be inhibited by necrostatin-1s (NEC1s) and/or necrosulfonamide (NSA) *in vitro*[27]. Several RIPK1 and RIPK3 inhibitors developed by Glaxosmithkline (GSK) have been studied in mice and Phase I/II trials in human but did not reach clinical phase III yet[40]. Interestingly, ponatinib (FDA-approved for chronic myeloid leukemia) and pazopanib (FDA-approved for advanced/metastatic renal cell carcinoma and advanced soft tissue sarcomas) were identified as TNF alpha-induced necroptosis inhibitors, unfortunately their clinical application is not promising due to their cardiotoxicity[41]. LY3009120, a pan-RAF inhibitor is also a necroptosis inhibitor and was shown to be a potential therapeutic drug for colitis in mice[42]. Various other compounds have been identified as inhibitors of necroptosis, including microRNAs, mixed lineage kinase domain-like protein (MLKL) inhibitors, heat shock protein (HSP) 90 inhibitors and even natural compounds of Chinese medicinal plants, but have not reached the clinic yet[40].

Besides pathway interference, the reduction or even removal of direct triggers of NET formation, such as ICx in SLE patients, would be a potential beneficial treatment strategy to reduce and target NET formation. In the SYNBIOSE-1 study, a combination of RTX and BLM was shown to largely decrease autoantibodies and NET formation, which also led to clinical benefit[36,43].

Of note, many NET-targeted compounds are tested in the setting of PMA or calcium ionophores-induced NET formation, which does not reflect the *in vivo* situation in AAV or SLE patients[29], and therefore should be interpreted with caution. Also studies in mouse models are also not always representing the human situation well, because there are important basic differences in their neutrophil-related immunity[44,45]. Unfortunately it is not guaranteed that successful NET targeting therapies *in vitro* or *ex vivo* have similar effects *in vivo*[46]. Therefore, longitudinal studies are needed to investigate the NET targeting potential of new and existing therapies in AAV and SLE patients, where quantification of NETs is performed in an unbiased manner in close relation to the *in vivo* situation.

There is a large amount of *in vivo* evidence for (neutrophil) extracellular DNA traps to have an important function in autoimmune diseases, host defense, cardiovascular disease, thrombosis and haemostasis, cancer and the development of metastases[47]. Lastly, NETs even have recently been reported to be involved in pathogenesis of the coronavirus disease 2019 (COVID-19)[48,49], which has caused a pandemic affecting millions of individuals resulting in severe health, social and economic crises world-wide.

B-cell targeted therapies in SLE

It is widely accepted that B-cells have a central role in the pathogenesis of SLE. Nevertheless. targeting B-cells with the anti-CD20 antibody RTX failed in two large RCTs[50,51], retrospectively due to incomplete B-cell depletion[52]. Of note, RTX does not directly target long-lived PCs, but causes depletion of their ($CD20^+$) precursors (i.e. B-cells and short-lived PBs)[36,53]. At the moment, only belimumab (BLM) has been FDA/EMA approved for the treatment of SLE patients[54,55]. BLM is an antibody targeting B-cell activating factor (BAFF), which is involved in the survival, proliferation and differentiation of B-cells[56]. Other strategies targeting B-cells or B-cell related pathways have been used off-label, including bortezomib that predominantly target long-lived PCs[57,58].

Despite the large amount of research and evidence pointing towards potential clinical benefit of targeting B-cells in SLE, the implementation of B-cell targeted therapies is not standard clinical care for SLE patients. It has long been known that SLE patients have increased frequency of plasmablasts/plasma cells that correlate with disease activity[59]. Therefore, there is a clear rationale for B-cell, and specifically plasma cell targeted therapy in SLE patients.

We demonstrated that monthly BLM after RTX (compared to RTX alone or BTZ alone) demonstrated the strongest reduction of ICx-mediated inflammation, including complement activation and NET formation, in severe SLE patients[60]. This was due to strong reductions of anti-C1q, high-avidity anti-dsDNA autoantibodies and decreasing the autoantibody repertoire which led to clinical benefit. These immunological effects were less pronounced for RTX and not seen for BTZ. Long term data on monthly BLM after RTX in SLE patients demonstrated that treatment did not have a long-lasting effect on plasma cells (which repopulated already after 24 weeks). In contrast, it rather inhibited repopulation of naive, double negative (DN) and memory B-cells, while all SLE patients kept suppressed autoantibody levels[43]. On the other hand, BTZ does predominantly target long-lived PCs and cause a significant depletion of $CD20^-$ PCs in peripheral blood (PB) and bone marrow (BM) in SLE patients, whereas their pre-cursor B-cells and T cells remained largely unaffected[57,58]. However, after BTZ withdrawal, a rapid repopulation of short-lived $HLA\text{-}DR^+$PCs, but not long-lived $HLA\text{-}DR^-$PCs, occurred, accompanied by increasing autoantibody levels[58].

Altogether these data implicate that in SLE patients the pathogenic culprit in the B-cell compartment responsible for autoantibody levels does not reside within the mature plasma cells, but rather in the naive, memory and specifically the DN B-cells compartment and their proliferation/differentiation into ASCs. These DN B-cells, also defined as $CD11c^{hi}$T-bet^+ $CD21^{low}$ $CD24^{low}$ $CD27^{low}$ $CD38^{low}$, were demonstrated to be expanded in SLE patients, present in the diseased kidney and correlated with disease activity[61-63]. This subpopulation is thought to be antigen experienced B-cells, despite low expression of CD27, and are able to differentiate in autoreactive plasma cells that produce SLE-specific autoantibodies upon T-cell, IL-21 and/or TLR7 stimulation[61-63]. Moreover, specifically these DN B-cells were reduced by RTX+BLM coinciding with significant reductions of anti-ENA, -dsDNA, and -C1q autoantibodies[43]. Recently, it was demonstrated that DN B-cells are expanded in multiple autoimmune and inflammatory neurologic diseases, such as multiple sclerosis (MS) and Guillain-Barre (GBS) syndrome[64].

Of interest, anti-ENA (i.e. RNP70, U1RNP, Sm) autoantibodies were previously shown to be stable over time and unresponsive to conventional treatment[65]. These anti-ENA autoantibodies were not significantly reduced by BTZ[57], while BLM after RTX strikingly did reduce these anti-ENA autoantibodies[43]. An interesting *in vitro* study demonstrated that isolated anti-ENA specific autoreactive B-cells (ABLs) in SLE were naive ($CD27^-$) activated B-cells which could differentiate *in vitro* into anti-ENA producing ASCs upon stimulation[66]. Another study showed that ANA^+ B-cells have a similar frequency in the transitional, naïve, memory B-cells and plasma cells of healthy subjects and SLE patients, while the frequencies decrease with maturation[67]. However, there was an absolute expansion of the ANA^+ IgG^+ plasma cells in SLE patients, possibly due to a generalized expansion rather than compromised tolerance checkpoints[68]. This further supports that the pathogenic culprit in SLE lies in the differentiation and proliferation of autoreactive naive, memory and DN B-cells towards ASCs[68]. Moreover, SLE patients have different phenotypes of ANA^+ antigen-experienced B-cells, reflecting an extrafollicular and a germinal center pathway leading towards autoreactive ASCs[69].

Altogether, autoreactive DN B-cells are a highly interesting biomarker in SLE patients, which should be considered and further studied when evaluating B-cell targeted therapies in SLE patients. Secondly, future studies should focus on identifying specific autoreactive (anti-dsDNA, anti-C1q, anti-ANA, anti-ENA) B-cells in relation to B-cell targeted therapy which will increase our understanding in the autoreactive B-cell compartment in SLE.

B-cell targeted therapies in AAV

In AAV, MPO- and PR3-ANCAs and the ANCA-producing B-cells have a central role in its pathogenesis[70-73]. It has been shown that targeting of ANCAs and B-cells with RTX, a B-cell depleting agent, is beneficial[9,10,17,19,74-76]. RTX is a registered first-line treatment for remission-induction and maintenance treatment in AAV patients[77]. In two RCTs, RTX was shown to be non-inferior to CYC as remission-induction treatment, which has been the golden standard for decades[9,10]. The safety profile of RTX was shown to be better than cyclophosphamide, specifically regarding the risk of developing a malignancy[15] and the risk of ovarian failure and male infertility[77]. In theory, (addition of) BAFF inhibition with belimumab could also be attractive in AAV patients[78]. Currently, a combination of RTX and BLM versus RTX and placebo is conducted in a phase II trial (COMBIVAS, NCT03967925).

The use of RTX as remission-induction regimen in AAV patients is associated with a relatively high rate of relapses[79]. After RTX remission-induction, 28% of the patients relapsed within two years without additional maintenance treatment[10,79].

Therefore, strict monitoring of patients is essential during the maintenance phase, after patients have reached remission. Recently, several randomized clinical trials demonstrated the superior efficacy of RTX as maintenance treatment[11,12,80]. The RITAZAREM study showed that 4 monthly fixed low-dose RTX was superior to AZA as maintenance therapy after remission-induction with RTX[81]. Additionally, the MAINRITSAN-3 trial showed that extended biannual RTX as maintenance for 18 months was superior to conventional maintenance therapy with corticosteroids[13]. Still, there is no consensus at the moment on the frequency, timing and dosage of RTX infusions for maintenance therapy, as studies have used different intervals and dosing (i.e. every 4 or 6 months, biomarker guided, with dosing ranges from 500 to 1000 mg).

Therefore, we need early biomarkers to guide (re)treatment that can predict and thereby potentially prevent relapses. Several studies have provided supporting evidence that ANCA and B-cell status could guide therapeutic decisions to prevent relapses in AAV patients after RTX as remission-induction regimen[19,75,82-84]. Importantly, our study indicates that both ANCA and B-cell status could guide therapeutic decisions to prevent relapses in AAV patients after RTX as remission-induction regimen. In addition, we demonstrated that a fixed RTX strategy will lead to overtreatment of a patients that achieved an ANCA-negative status and have a low risk of relapse. MAINRITSAN-2 trial demonstrated that ANCA and B-cell guided RTX reached similar relapse frequencies as fixed dosing of RTX, while using less infusions[11]. Nevertheless, it should be taken into account that also ANCA- and B-cell-tailored RTX maintenance could lead to overtreatment because 37-58% of patients with ANCA positivity and/or B-cell repopulation relapsed. Given the limitations of several retrospective studies on this issue, future studies are warranted to evaluate the added-value of these biomarkers in a prospective study to establish whether ANCA and B-cell immunomonitoring could actually reduce overtreatment and damage accrual in AAV patients. Of note, the choice of the remission-induction regimen (RTX vs CYC) will influence the biomarkers (ANCA and B-cells), which should be kept in mind during study design[9,83]. Importantly, the sensitivity of the method used to analyze B-cells after RTX determines the detection level of B-cell depletion and also their reconstitution[85].

One could argue that remission-induction with RTX by itself is not effective enough and therefore combining RTX and CYC could be beneficial. CYC has a broader effect on the immune system and inhibits next to B-cells also $CD4^+$ and to a lesser extent $CD8^+$ T cells[86]. Actually, three cohort studies have already demonstrated that the combination of RTX with CYC resulted in clinical remission with a favourable immunological state and the ability to rapidly taper corticosteroids[76,87,88]. 52% of the patients that received the combination of RTX+CYC reached ANCA-negativity within 6 months[76], in comparison to

23% in our RTX-treated cohort. The combination even had significantly lower relapse rates than a matched control cohort group[76]. Moreover, glucocorticoids (GC) could rapidly be tapered after RTX+CYC which was associated with reduced GC-related adverse events[88]. These studies showed that the combination of RTX and CYC was feasible and prolonged B-cell depletion was not associated with an unexpectedly high incidence of adverse events. Based on these insights we hypothesized that combination of RTX with low dose CYC will lead to more achievement of ANCA-negativity, prolonged B-cell depletion and less relapses on the long term. This will be further studied in the ENDURRANCE study (NCT03942887), a randomised controlled trial for AAV patients aimed to compare RTX versus RTX+CYC in a controlled prospective setting, where after patients will receive tailored maintenance RTX based upon ANCAs and B-cells.

The observation that relapses occur frequently after RTX, suggests that minimal residual autoimmunity (MRA) resides in the B-cell compartment in AAV patients. Indeed, we demonstrated that despite significant reductions in circulating B-cell numbers after RTX, B-cells always remained detectable when employing Euroflow-based HSFC[89]. This is clinically relevant because AAV patients with residual B-cells (≥1 x 10^6 B-cells/L) after RTX, had significantly more relapses[90], in line with another study[91]. Additionally, the return of B-cells after RTX has also been recognized as a risk factor for relapse[17]. However, patients can also relapse without detectable B-cells (below the conventional threshold of flow cytometry)[11,92,93].

Different studies have shown that specific B-cell populations have a distinct pathogenic role in AAV disease. Recently, $CD27^+CD38^{++}$ plasma cells were shown to be increased at baseline in patients that relapsed in the future[94]. The repopulation of naive B-cells after RTX at 6 months was associated with a reduced risk of relapse[91]. Also, regulatory B-cells (Breg) have been described as a key B-cell subpopulation responsible for maintaining self-tolerance[95]. Indeed, these Bregs, present among $CD5^+$ B-cells, inversely correlated with disease activity in AAV patients after RTX[96,97]. We demonstrated that DN B-cells had the strongest association with ANCA levels[89]. Only one other study describes DN B-cells in AAV, showing that at baseline AAV patients had significantly higher proportions of DN B-cells than HCs[98]. Moreover, AAV patients with an increased proportion of class-switched memory B-cells or DN B-cells had higher BVAS scores at 6 months, while there was no association between plasmablasts and disease activity.

Altogether, the memory and specifically the DN B-cell compartment is an interesting B-cell subset in AAV which should be further evaluated. In line with this, future studies are warranted to better assess MRA and its added value to associate with disease activity or relapses in AAV patients.

CONCLUSION AND FUTURE PERSPECTIVES

In this thesis we aimed to gain more insight on the role of NETs, autoantibodies and autoreactive B-cells in the pathogenesis of AAV and SLE. Moreover, we aimed to understand the humoral autoimmune response and to identify targets for immunomonitoring in the setting of B-cell targeted therapies. Armed with this knowledge we will be able to further improve targeting of autoimmunity in AAV and SLE patients and advance their clinical care.

Our studies demonstrate that NETs have a pivotal role in both AAV and SLE patients. NETs function as autoantigens, can cause direct glomerular inflammation and can be part of immune-complexes in SLE. Importantly, AAV and SLE-induced NETs are disease-specific processes that each encompassed their own unique properties. This should be taken into account when evaluating targeting of NETs in AAV and SLE. In SLE patients, NETs could be targeted through reducing the autoantibody repertoire, specifically high-avidity anti-dsDNA and anti-C1q autoantibodies that drive immune complex formation. These autoantibodies were effectively targeted by combined treatment with RTX and BLM. The exact triggers of NET formation in AAV are not completely known, taken into account conflicting studies on the role of ANCAs in NET formation. During B-cell targeted therapy in AAV and SLE patients, the presence and reoccurrence of autoreactive B-cells and relevant autoantibodies are components of minimal residual autoimmunity (MRA), which often persists after B-cell therapy.

Interestingly, both in AAV and SLE, double negative (DN) B-cells have a key role in the humoral autoimmune response and were associated with reoccurrence of autoantibodies. However, it remains to be established how MRA is associated with disease flares and to find the best way to use it as immunomonitoring tool to guide and personalize treatment. Altogether, our studies clearly demonstrate that investigating the sources that drive autoantibody formation captures a more precise reflection of humoral autoimmunity in AAV and SLE patients. Future studies should focus on identification of disease-related NET triggers and pathways involved in AAV and SLE-induced NET formation. Additionally, studies should focus on targeting DN B-cells in both SLE and AAV and investigate their dynamics in the light of B-cell targeted therapies. Lastly, the identification of autoantigen-specific B-cells will possibly lead to increased understanding of the pathogenesis of AAV and SLE.

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