Review

MOLECULAR METABOLISM

Metabolic syndrome – Removing roadblocks to therapy: Antigenic immunotherapies*

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ABSTRACT

Up to 25 per cent of the world's adult population may have the metabolic syndrome, a condition closely associated with central obesity. The metabolic syndrome is a major risk factor for cardiovascular disease and type 2 diabetes and therefore represents an important worldwide health problem. In addition to metabolic abnormalities such as raised fasting plasma glucose, high cholesterol and high blood pressure, there is consensus that obese subjects develop a state of low-grade chronic immune activation. This sustained pro-inflammatory response in fat tissue is thought to worsen insulin resistance and dyslipidemia. Likewise, the immune system contributes to the detrimental cascade of events leading to plaque formation in atherosclerosis. It has long been assumed that the innate arm of the immune system was the only key player, but emerging evidence suggests that there is in fact a sizeable adaptive immune component to obesity and cardiovascular disease. From a therapeutic perspective, it could be envisioned that immune modulation drugs such as cytokine inhibitors, co-stimulation blockers or anti-T cell agents could offer benefit. It is questionable, however, whether chronic treatment with for instance biologicals will have a favorable risk/benefit profile in a silent condition such as the metabolic syndrome. An attractive alternative could be the development of antigen-specific T cell therapies, not unlike those currently in various phases of development for type 1 diabetes. In this article, we will give an overview of antigen-specific treatment modalities in type 1 diabetes, followed by a review of the evidence for T cell involvement in obesity and atherosclerosis.

Keywords Metabolic syndrome; Type 1 diabetes; Type 2 diabetes; Obesity; Atherosclerosis; T cells; Macrophages; Antigenic immunotherapies

1. INTRODUCTION – THE AUTO-INFLAMMATORY COMPONENT IN OBESITY AND ATHEROSCLEROSIS

The adaptive immune system protects us on a daily basis from cancer cells, fungi, viruses and bacteria. Its primary cellular components, B and T lymphocytes, carry a repertoire of highly diverse antigen receptors that allow for the efficient discrimination between self and foreign substances. In order to ensure that no self-reactive T cells are released into the periphery, a stringent selection process exists in the thymus. Even when thymic selection fails and a potentially destructive, tissue-specific T cell clone enters the circulation, powerful backup mechanisms come into play such as those mediated by natural regulatory T cells (nTreg). In healthy individuals, these complex control mechanisms collectively ensure that immune homeostasis is permanently maintained.

Similarly sophisticated control mechanisms are in place to avoid survival and proliferation of cells with a faulty cell cycle. Yet we know that in subjects with a specific genetic background, escape of a single cancer cell from surveillance can have dramatic consequences. Likewise, escape of a single T cell from thymic selection in genetically susceptible individuals can lead to recognition and destruction of its tissue target, eventually culminating in autoimmune disease [1]. Depending on the reactivity of the autoreactive T cell subset, a wide spectrum of conditions arises, including common diseases such as rheumatoid arthritis, Crohn's disease or type 1 diabetes. Apart from a few exceptions such as pemphigus vulgaris, myasthenia gravis and autoimmune gastritis, we still do not know what the inciting autoantigen is in most autoimmune diseases.

In the last decades, remarkable progress was made in the treatment of many of these conditions. We may not always know precisely what causes autoimmunity, but our understanding of the effector mechanisms has greatly improved. Taking rheumatoid arthritis as an example, this once debilitating disease is now managed extraordinarily well in most patients owing to the availability of a host of biologicals. These drugs often tackle distinct components of the innate (anti-TNF, IL-1, IL6) or adaptive (anti-CD20 therapy, CTLA-4Ig) immune system. An essential drawback of virtually all of these immune modulators is that they do not act specifically enough to fully preserve critical host defense mechanisms. For instance, anti-TNF class agents carry a black box warning for potential malignancy risk and are associated with increased susceptibility to bacterial infections. This side effect profile may be acceptable in treatment of severe inflammatory diseases

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such as rheumatois arthritis and Crohn's disease that occur primarily in adults. It is, however, unlikely to become standard practice in conditions such as type 1 diabetes, with a considerable pediatric population and an excellent prognosis with optimized exogenous insulin therapy.

It is thought that the cause of autoimmune disease is that the immune system at some point encounters self-antigen in the wrong microenvironment [2]. A hypothetical example is that of an autoreactive T cell entering a tissue that is affected by viral infection. Here, the T cell will recognize its tissue antigen in the presence of danger signals originating from the ongoing viral infection. The result will be activation followed by autoimmune tissue destruction. In the face of that scenario, it was postulated that the reverse mechanism could be the path to restoring balance, i.e. by presenting the antigen in such a way that T cells are 're-educated' and start 'seeing' the tissue antigen as self again [3]. One such pathway is through administration of antigen via the oral route, a mechanism termed oral tolerance [4]. The concept entails that the default immune response toward food antigens is tolerogenic, and it is a remarkably well documented phenomenon in animal models for autoimmune disease. The potential benefits over non-antigen specific immune modulators are significant in the sense that one targets only the disease-relevant immune cells, thereby avoiding the deleterious consequences of immune suppression.

Antigen-specific therapy may also hold promise in the treatment of the metabolic syndrome and its cardiovascular consequences. It is now widely accepted that low-grade chronic inflammation at least contributes, if not drives, insulin resistance and dyslipidemia in obesity [5] and plaque formation in atherosclerosis [6]. More recently, it was shown that adaptive immunity plays an essential part in this process. Whether obesity and atherosclerosis are true autoimmune diseases in origin is still questionable, but they definitely appear to contain an auto-inflammatory element. While the nature of the relevant antigen(s) is still entirely unknown in obesity, some proof-of-concept studies show that oral tolerance induction against disease relevant antigens works against atherosclerosis. The safety profile and simplicity of antigen-specific therapy seem tailored to treatment at onset of the metabolic syndrome, a disease stage during which chronic treatment with expensive immune modulators is not advisable. The ambition should be to design a safe antigenic immunotherapy that can be administered from diagnosis of the metabolic syndrome in order to neutralize the obesity-related immune component and/or prevent cardiovascular disease.

2. ANTIGEN-SPECIFIC THERAPIES IN TYPE 1 DIABETES

Type 1 diabetes (T1D) is a prototypical autoimmune disease that is characterized by the specific destruction of insulin-secreting beta cells in the pancreas by the immune system [7]. Like virtually all other autoimmune diseases, T1D is thought to be the consequence of a complex interplay between genes and environment. The specificity of the T cell repertoire against pancreatic islet antigens is fairly well-described in T1D, which makes that antigen-specific treatments are regarded as an attractive approach. In mice, insulin is a primary autoantigen [8], although other non-beta cell restricted molecules including GAD, IGRP and chromogranin are also targeted [9]. Human histopathology studies indicate that CD8 T cells are the predominant subset within islet lesions, react against various islet antigens and can be reliably identified in the periphery [10,11] (Fig. 1).

T1D is in essence a curable autoimmune disease, as evidenced by immunosuppression trials decades ago [12] and more recently by non-myeloablative stem cell transfers [13]. Two substantial limitations, however, impede broad application of these therapies. First and foremost, the side effect profile associated with these therapies is generally unacceptable given the current high standard of care with exogenous insulin therapy [14,15]. Second, the effect of C-peptide preservation is not lasting, meaning that treatment should be administered chronically which, in view of the aforementioned side effects, is not an option. The same objections largely apply to the use of islet transplantation, where islet grafts are eventually lost due to recurring autoimmunity in the absence of adequate immune modulation [16].

Antigen-specific therapies offer a potential solution to these challenges [17]. The concept entails presentation of key autoantigens to the immune system in such a way that they are viewed as 'tolerogenic' and, as a consequence, immune ignorance or regulation is restored. One of the most attractive features of antigenic therapies is that only the disease-relevant part of the immune system is silenced, while protective immunity is left intact. This attribute could allow for safe chronic treatment regimens, even in a secondary prevention setting in autoantibody-positive, at-risk non-diabetics.

In animal models, antigenic treatment has shown great promise in preventing disease but has never been reliably capable of disease reversal, something that immune modulators such as anti-CD3 were able to achieve [18]. Therefore, antigenic treatments could be envisioned as a suitable follow-up therapy after a short-course

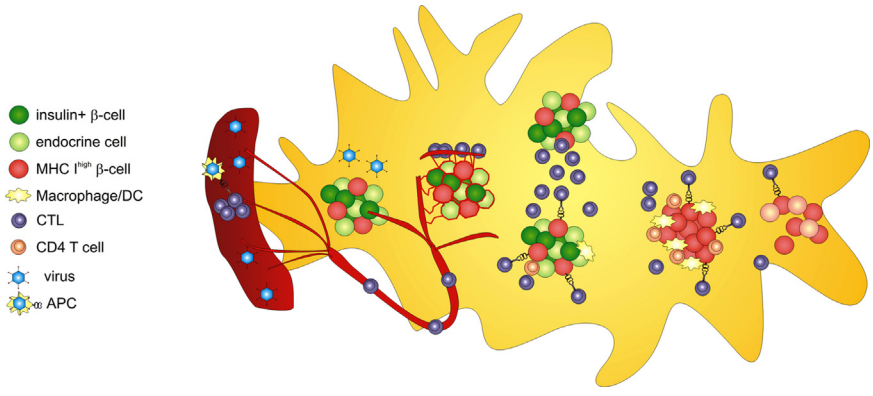

Figure 1: The autoimmune process in T1D. The figure outlines some key steps in the development of T1D, from left to right. It is assumed that an environmental trigger, for instance a virus, triggers the activation of certain autoreactive T cells that have escaped thymic selection. Whether the infection needs to target the target organ for disease induction is unknown. Next, the activated T cells, primarily CD8 T cells (CTL) in T1D, extravasate and accumulate around the pancreatic islets. There, they recognize their cognate autoantigen presented by MHC class I on beta cells and by APC's such as macrophages, resulting in proliferation and cytokine release. Cytokines and cell-mediated killing eventually result in profound inflammation and beta cell death.

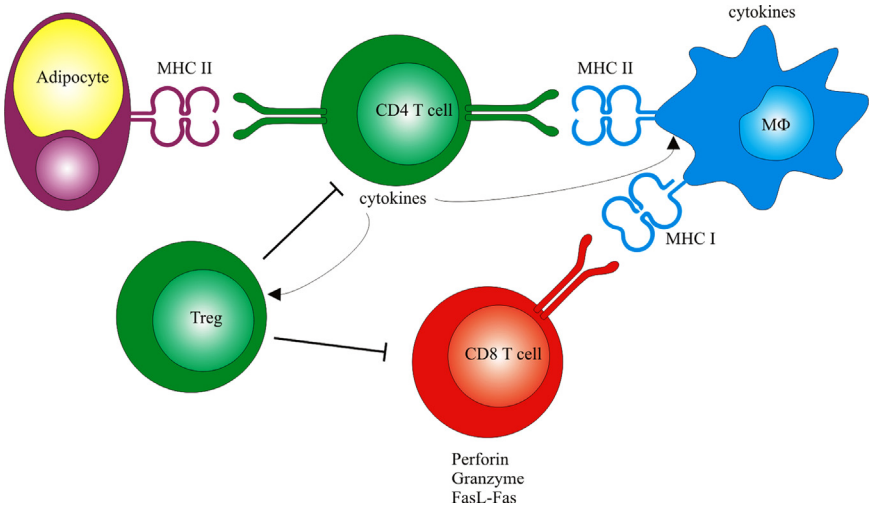

Figure 2: The adaptive immune system in obesity. In adipose tissue, CD4 T cells are activated by either macrophages or directly by adipocytes, which present an unknown antigen in the context of MHC class II. It is possible that NKT cells, an innate-like T cell subset, may be of particular importance since these cells specifically recognize lipids. This activation will trigger cytokine release, primarily IFN-γ, which in turn promotes conversion of M2 macrophages to a pro-inflammatory M1 phenotype. These M1 macrophages produce copious amounts of innate cytokines such as TNF-α and IL-1β, which are known to promote insulin resistance and enhance tissue inflammation. Regulatory T cells, which under steady state conditions secure immune homeostasis, are affected by the proinflammatory milieu and fail to regulate the autoreactive process. CD8 T cells also appear to be involved and enhance the recruitment of macrophages, resulting in a vicious cycle of inflammation.

treatment with an immune modulator such as anti-CD3. Data in mouse models confirm that this indeed may be a viable option in order to avoid the long-term side effect profile associated with chronic immune modulation [19].

Despite encouraging evidence in animal models, the translational record of antigenic therapies remains checkered. As a monotherapy, these approaches may lack the therapeutic potency required to reverse what is essentially an end-stage disease phase at diagnosis. Whereas combination therapies sound like an attractive alternative, there are significant regulatory and commercial impediments that currently restrict their clinical translation [20]. We will outline below a selection of successes and failures with antigenic therapies in T1D, which could conceivably also be tested in the metabolic syndrome. Importantly, emerging evidence suggest that antigenic treatments for T1D could also potentially be applied in subsets of T2D patients, as overlapping beta cell-specific CD4 T cell reactivity profiles are observed in both conditions [21].

2.1. Mucosal antigen delivery

Perhaps the most straightforward antigen delivery method is via the oral route. This approach exploits the notion that the default immune response to food antigens is of tolerogenic nature. This idea dates back to 1911 and originated from experiments where hen's egg protein was fed to guinea pigs, rendering them refractive to injections with the antigenic protein [22]. Recent clinical studies in the area of food allergies to eggs [23] and peanuts [24] have suggested that high-dose oral feeding regimens can induce a certain degree of desensitization. Whether this amounts to the establishment of lasting tolerance is still unclear, although the most recent egg allergy trial showed prolonged desensitization in about a quarter of patients [23].

In the field of autoimmunity, the concept was rediscovered by Weiner and colleagues and has shown remarkably consistent efficacy in a variety of animal models for T1D [25], multiple sclerosis [26], arthritis [27] and others. The protective effect is typically achieved in mice by repeated oral feeding of low milligram doses of soluble protein prior to disease onset. In the spontaneous NOD model, treatment with oral insulin has to be initiated early at 5 weeks of age and weekly feeding is maintained throughout the course of observation. In this model, both the source of insulin and dose appear to significantly influence the outcome [28]. An encouraging finding is that insulin feeding not only established tolerance against insulin, but also other islet-specific antigens by virtue of a mechanism coined 'bystander suppression'. This is important because the ambition in T1D is to prevent the destructive activity of all autoreactive T cell species, not just those specific for insulin [29].

The approach has been tested in the clinic in a series of autoimmune conditions, so far with disappointing outcomes [3,30]. Of note, most trials, including some in T1D, have treated diagnosed individuals. This setting contrasts with the findings in animal models, where solid effects are only observed in a prophylactic treatment course. The DPT-1 trial in at-risk, non-diabetic relatives from T1D patients raised high hopes but unfortunately failed to meet its primary endpoint [31]. Subgroup analysis identified an effect in insulin autoantibody positive individuals, which serves as an inclusion criterion in an ongoing prevention trial.

Nasal administration of autoantigens is an alternative route of mucosal delivery. It has been argued that this pathway is preferable because of the lack of gastrointestinal antigen degradation and, in the NOD mouse, this approach indeed conferred protection [32]. Given that mucosal antigen delivery by itself may not be sufficient to cure established disease, combinatorial methods could be an option. Combination of an anti-T cell agent, anti-CD3, with oral [33] or nasal [19] (pro-)insulin has shown great promise in various animal models. This type of treatment course reduces the risks associated with long term immune suppression, increases efficacy by means of synergy and could be an excellent pathway to explore in the metabolic syndrome.

2.2. Parenteral 'vaccination' in suitable 'adjuvant'

The concept of therapeutic vaccination with autoantigens in autoimmune disease may sound paradoxical at first. A relevant example of how injected antigens can tolerize against the effects of later exposure is found in bee keepers. A recent publication showed that these individuals react normally against the first bee stings of the season but are soon tolerized and show desensitization throughout the rest of the season [34]. Mechanistically, this reduced response is associated with a functional T cell switch away from Th2 immunity toward a Tr1-mediated regulatory response. An intriguing finding is that desensitization only lasts as long as

the antigen exposures, i.e. by the start of the next season all bee keepers are again sensitized and T cell functionality returns back to baseline. This observation could indicate that antigenic injections can only prevent autoimmunity through sustained treatment.

The most advanced agent within this category is DiaPep277®, a modified peptide derived from HSP60, formulated in vegetable oil and administered subcutaneously. This drug induces immunological tolerance through antigen-specific and non-specific (TLR-2) signaling, which recently resulted in promising Phase III outcomes in recently diagnosed T1D patients [35]. Another example is Diamyd®, GAD65 formulated in alum adjuvant, a classic T1D-associated autoantigen. Whereas Phase 2 trials suggested that the drug had regulatory capacities and could preserve C-peptide, two recent Phase 3 trials failed to achieve their endpoints [36,37]. Finally, the parenteral arm of the DPT-1 trial, referenced above, treated at-risk subjects with insulin injections with the aim of correcting metabolic and immunological imbalances prior to diagnosis [38]. Unfortunately, this trial failed to prevent diabetes development.

Thus, despite promising pre-clinical data and some evidence of efficacy in Phase 2 trials, it appears that our incomplete understanding of variables such as dose, formulation and regimen may hinder optimal clinical translation of antigenic therapies. Conceivably, similar challenges may need to be overcome in developing antigen-specific therapies for the metabolic syndrome.

2.3. DNA-encoded delivery

An alternative antigen delivery method stems from the observation that injected DNA sequences in the form of plasmids can induce protein expression in mouse muscle cells in vivo [39]. After injection, the plasmids are rapidly taken up by the local muscle cells which produce the proteins encoded by the plasmid. Whereas most applications of this concept have been directed at immunization against infectious diseases, this type of antigen delivery has also shown promise in a T1D setting [40]. The advantages over protein-based antigen delivery are the low cost in combination with prolonged and targeted antigen delivery. Phase 2 trials were completed in MS [41] and T1D [42] and collectively show that DNA vaccination is a safe approach with some evidence of efficacy. In the T1D trial it was shown that certain disease-relevant CD8 T cell species can be specifically eliminated, a mechanism that could possibly translate into a highly targeted treatment in the metabolic syndrome as soon as the driving antigens are discovered.

2.4. Nanoparticle-coupled delivery

An emerging new avenue that holds potential to improve the efficacy of antigenic treatments involves coupling of autoantigens to nanoparticles. A substantial body of pre-clinical data suggests that peptide-coupling to apoptotic splenocytes can safely induce antigen-specific tolerance [43,44]. However, this approach is difficult in terms of adherence to good manufacturing practice standards. Nanoparticles, used as inert surrogate cells, could be implemented as drug delivery vehicles in order to deliver antigenic peptides. The recent work shows that such antigen-loaded nanoparticles can indeed induce durable immune tolerance in animals with autoimmune disease [45].

3. THE IMMUNE SYSTEM IN OBESITY

Antigen-specific therapy in T1D clearly holds promise, mainly owing to its low risk for side effects in a vulnerable patient population with excellent treatment alternatives. In order to treat obese patients early in order to avoid later inflammation-associated complications, diagnosis of the metabolic syndrome could be a viable initiation stage for antigen-specific intervention. An excellent safety profile is a prerequisite. In view of efficacy, an antigen-specific inflammatory component to the disease needs to be established. Although the metabolic syndrome is often associated with an increased inflammatory immune status, this has generally been considered a secondary rather than causative phenomenon. Recent data, however, indicate that inflammation might in fact come first and may be a process that drives or at least contributes to insulin resistance.

3.1. Innate adipose tissue immunity

It is now widely acknowledged that the immune system in obese individuals adopts a state of chronic, multi-organ low grade inflammation. It is also believed that these inflammatory imbalances, characterized for instance by circulating cytokines, are related to metabolic dysfunction [5]. Macrophages are part of the innate immune system and are normally involved in homeostatic phagocytosis, serving as scavengers for cellular debris. During host defense against infectious disease, macrophages are instrumental in the first line defense by means of non-antigen specific engulfment of pathogens. Finally, macrophages are professional antigen-presenting cells and are able to initiate and direct adaptive T cell responses. Macrophages can be found throughout the body under physiological conditions, including in adipose tissue. In lean individuals, their functional phenotype in adipose tissue is described as 'M2', or alternatively activated macrophages. This subtype generally suppresses inflammatory responses. In obese subjects, however, the predominant macrophage subtype is of the 'M1', classically activated type, which is associated with proinflammatory stimuli. The precise signaling cascade involved in M2-to-M1 transition has not been elucidated, but some key pathways have been identified. Eosinophils appear to play an essential role in the maintenance of macrophages' M2 phenotype in lean adipose tissue [46].

An important notion is that some of the pattern receptors used in pathogen sensing also recognize certain endogenous molecules that are enriched within fat tissue. Examples include Toll-like receptors (TLR) [47] and NOD-like receptors (NLR) [48]. Interestingly, these pivotal pattern receptors are also known to interact with the gut microbiota, and disturbances within this cross-talk can promote the metabolic syndrome [49]. The action of macrophages affects many other tissues in obese subjects such as muscles, blood vessels and notably, as in type 1 diabetes (T1D), causes beta cell destruction in the pancreas. Whereas in T1D-associated islet lesions T cells dominate, T2D-associated islet infiltrates consist predominantly of macrophages [50]. Finally, the involvement of innate immune mechanisms in T2D is suggested by clinical trials blocking activity of typical macrophage-associated cytokines such as IL-1β. The beneficial effects in terms of improved glycemia, beta-cell function and downregulated systemic inflammation markers indicate that macrophage-driven inflammation may be a potent disease driver in T2D [51].

3.2. Adaptive immunity and Tregs in adipose tissue

T cells have only recently been discovered to play a role in adipose tissue inflammation. T cells are more abundant in adipose tissue from obese mice as compared to their lean counterparts and actually arrive before macrophages start accumulating [52]. Given that the cytokines that dictate the balance between M1 and M2 macrophage phenotypes are typical T cell-derived cytokines, their simultaneous influx would seem logical. The T cell profile in adipose tissue indeed undergoes several important changes, one of which is an increase in the CD8/CD4

T cells ratio long before adipose tissue macrophage influx [53]. Furthermore, absence of CD8 T cells abrogates macrophage infiltration, while enrichment has the opposite effect. An obvious candidate T cell subset that could account for local suppression of adipose tissue T cells are natural regulatory T cells (Treg). It was indeed shown that with proceeding obesity, adipose tissue Treg numbers progressively [54,55] (Figure 2).

Do these observations suggest that antigenic therapies may bear future potential in treating or preventing adipose tissue inflammation in obesity? While no antigens have been identified that can directly activate T cells in adipose tissue, there are encouraging signs that cognate adipose tissue antigens exist and are locally presented. First, the T cell repertoire in obese adipose tissue is remarkably restricted, hinting towards the possibility of antigenic selection in response to certain adipose tissue-derived autoantigens [54,55]. Second, evidence exists that the essential event leading up to T cell activation, antigen presentation within a MHC context, actively occurs within obese adipose tissue. Whether it is primarily macrophages [56], B cells [57] or adipocytes [58] that perform the bulk of the local antigen presentation is still under debate. Third, immunotherapy with anti-CD3 antibodies, arguable the most successful treatment in mouse models for T1D, improves Treg control and insulin resistance [55]. This suggests that immunotherapy in obesity can have meaningful benefits that could may be optimized by means of combination therapy with antigen, a strategy that also shows potential in T1D [19]. Finally, although data are currently scarce, it may be that tolerance mechanisms are inherently affected by the obese state, as some oral tolerization studies suggest [59]. A recent animal study suggests that high-fat diets promote intestinal absorption of gut antigens, which in turn leads to delivery of the antigens to adipose tissues via chylomicrons [60]. The possibility therefore exists that the adipose tissue T cells in obese subjects recognize an exogenous rather than an endogenous molecule. This would obviously constitute a major impediment to antigen-specific tolerance induction, as each patient would harbor an unpredictable adipose tissue T cell repertoire.

How do we proceed with defining the specificity of adipose tissue-associated T cells? The area of epitope discovery is well-developed in T1D research, with several major autoantigens being characterized and inventoried in the Immune Epitope Database (IEDB) [61]. The conventional approach to identify the specificity of T cells involves reducing the complexity of the T cell population being screened (e.g. by T cell cloning), or selecting a limited set of candidate antigens for testing against a complex T cell mixture. Since this is a low-throughput procedure, future technological advances should provide discovery platforms aimed at more rapidly screening complex peptide mixtures against mixed T cell populations [62]. For protein antigens these high-throughput approaches should be guided by our detailed understanding of the adipocyte proteome [63]. The complexity of the peptide mixture to be tested can be substantially reduced by applying computer algorithms to predict epitope binding to the relevant MHC class II molecule [64]. In a recent atherosclerosis study by Tse et al., the 'candidate antigen' approach in combination with in silico prediction of MHC Class II peptide binding motifs was pursued. Here, two antigenic murine ApoB-100 peptide fragments were identified, and their binding to I-Ab determined by affinity assay. Utilizing a vaccination scheme in Apoe−/− mice, these antigens were shown to ameliorate plaque formation in vivo [65]. A similar stepwise approach could be envisioned in obesity models.

Searching for the antigenic trigger for adipose tissue T cells, one naturally arrives at an innate-like lymphocyte subset that is known to react to glycolipid antigens, the natural killer T (NKT) cell. Indeed, recent data show that adipocytes can modulate iNKT cell function. In an analog fashion as with MHC class II, adipocytes serve as lipid antigen-presenting cells in a CD1d-mediated fashion [66]. Under obese conditions, however, NKT cells leave the scene before macrophage accumulation start [67,68]. Taken together, these findings suggest that under lean conditions, adipose tissue-resident iNKT cells maintain local immune homeostasis through direct engagement of lipid antigens, presented by adipocytes. Antigen-specific treatment could thus consist of specific NKT cell lipid antigens, be it natural or artificial. In support of this hypothesis, in vivo activation of iNKT cells via an exogenous super ligand, alpha-galactosylceramide, decreased obesity-related metabolic parameters and inflammation [67].

In conclusion, adaptive immunity and T cell infiltration in particular appear to be early events in the development of adipose tissue inflammation in obesity. Whereas the field has not yet advanced to the identification of specific lipid autoantigens, there are strong indications that there is an autoinflammatory component to the condition. Identification of these antigens may initiate the application of antigenic therapies in a similar fashion as outlined within the context of T1D.

4. THE IMMUNE SYSTEM IN ATHEROSCLEROSIS

4.1. Innate endothelial immunity

Much like obesity, atherosclerotic disease was long seen as a lipid storage disease. Both diseases are now recognized to share another common pathophysiological property, which is inflammation [6]. Elevated levels of the inflammatory marker high-sensitivity C-reactive protein (hs-CRP) are associated with increased risk for both CVD and diabetes [69]. Whereas in obesity, free fatty acids can trigger inflammation, oxidized LDL has a similar pro-inflammatory effect in atherosclerosis. The molecular underpinning of atherosclerosis involves circulating LDL particles that penetrate the arterial wall, where they undergo oxidation. Oxidized LDL subsequently initiates endothelial cell activation which leads to expression of adhesion molecules and chemokines and eventually recruitment of macrophages. The functional macrophage phenotype cannot be clearly categorized as either M1 or M2, as is the case in adipose tissue inflammation [70]. These macrophages take up oxidized LDL particles via their scavenger receptors and may turn into so-called foam cells. The continuous influx of macrophages – and T cells, see below – followed by inefficient clearance of dead cells eventually culminates into the formation of atherosclerotic plaques. Evidence of innate immunity is further inferred from plasma markers such as TNF and IL-6, innate-like cytokines which are known to be important mediators of atherogenesis. Finally, macrophages may play a crucial role during the advanced stages of atherosclerosis leading up to acute vascular events. The unstable lesions that are ultimately responsible for releasing the thrombogenic material into the lumen, typically harbor a substantial amount of macrophages [70]. It is however still unknown how precisely macrophages contribute to the development of these culprit lesions.

4.2. Adaptive immunity and Tregs in the atherosclerotic plaque

It is generally assumed that T cells arrive in atherosclerotic plaques later than macrophages, probably as a result of antigen drainage to the lymph nodes [71]. As with macrophages, chemoattractants play a major role in the gradual accumulation of T cells in the lesions. It is clear that T cells in atherosclerotic plaques exhibit a more activated phenotype as compared to their counterparts in the periphery [72]. As in obese fat tissue, infiltrating T cells predominantly display a proinflammatory Th1

phenotype, characterized by IFN-γ secretion, which is a potent proatherogenic mediator. The precise roles of Th2 and Th17 subsets are still under debate [71] as is that of NKT cells [73]. Treg cell deficiencies are clearly involved in the pathogenesis, as Treg are detected in much lower amounts in atherosclerotic plaques than in other chronically inflamed tissues [74]. Moreover, adoptive transfer studies in animal models show that natural Treg are potent inhibitors of atherosclerosis [75].

Given that the presence of T cells in atherosclerotic lesions is firmly established, the question arises which antigens these T cells react to. Unlike for adipose tissue T cells, a number of T cell antigens have been defined in atherosclerosis. T cells isolated from atherosclerotic plaques reveal specificity for oxidized LDL [76] and may possibly recognize heat shock protein (HsP) 60 [77,78]. As a result of these findings, antigen-specific, tolerogenic vaccination against atherosclerosis is regarded as a promising treatment modality [79]. We will not cover strategies aimed at inducing active vaccination to neutralize key pathogenic proteins but rather focus on a few promising approaches that elicit active T cell tolerance.

There is firm evidence that tolerance induction against oxidized LDL is a feasible and effective approach in animal models. Atherosclerosis-prone mice given oral doses of oxLDL develop less atherosclerotic lesions, an effect that is accompanied by an increase in Treg and regulatory cytokines [80]. Related work focused on ApoB100, the core protein of LDL, which is a driver T cell target in mice [81]. Tolerogenic DCs pulsed with ApoB100 reduced the autoimmune response against low-density lipoprotein, and consequently inhibited atherosclerotic lesions in the aorta [82]. Subcutaneous infusion of ApoB100 peptides also reduces atherosclerosis in ApoE knockout mice through Treg stimulation [83]. Analogous data were acquired using oral or nasal administration of HSP65, with significant amelioration of macrophage and T cell infiltration and reduced plaque size [84,85].

Thus, in contract to the obesity-related inflammatory state, T cell antigens have been identified in atherosclerosis and pre-clinical studies suggest that T cell tolerization strategies hold promise.

5. CONCLUSIONS: TOWARD ANTIGEN-SPECIFIC THERAPY IN THE METABOLIC SYNDROME

Progress in identification of T cell antigens in T1D has enabled the design of antigen-specific therapies, aimed at reinstating immune tolerance. Pre-clinical data overwhelmingly demonstrate that these antigenic treatments are safe and effective, yet clinical translation has been problematic so far. We reviewed here a few notable examples of antigenic therapies that are in various stages of development, in order to point out some of the mechanisms, benefits and pitfalls associated with the concept.

The metabolic syndrome is primarily caused by central obesity and comprises a range of abnormalities including insulin resistance, dyslipidaemia, and hypertension, which are key risk factors for type 2 diabetes and cardiovascular disease [86]. Whereas the optimal solution remains weight reduction, safe therapeutic interventions may aid in avoiding some of these severe outcomes. While obesity and atherosclerosis were previously seen as lipid storage disease, it is now well established that both conditions share the involvement of adaptive immune mechanisms. Especially in atherosclerosis, antigen-specific T cell responses have been identified, enabling pre-clinical proof-of-concept studies using antigen-specific approaches.

We argue that diagnosis of the metabolic syndrome may be the ideal initiation stage for antigenic treatment, once well-characterized antigens have been defined in human disease. One could envision a safe chronic treatment with obesity- and/or atherosclerosis-associated antigens to break the vicious cycle of inflammation in adipose tissue and/or atherosclerotic lesions. The health benefits of abolishing the inflammatory component could obviously be substantial. Lessons from oral tolerance experiences in T1D teach us that antigenic treatment needs to be initiated as early as possible, preferably before overt disease, and thus diagnosis of the metabolic syndrome may be a suitable phase.

CONFLICT OF INTEREST

KTC and MGvH are both employed by Novo Nordisk.

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