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Perspective

Immune therapies for autoimmune diabetes targeting pathogenic peptide–MHC complexes

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At present, there is no cure for type 1A diabetes (T1D), a T cell-mediated autoimmune disease. Monoclonal antibodies (mAbs) are used to treat a wide number of diseases. For treating T1D, mAbs that target major immune cell subsets show considerable promise, but so far, when used at doses that do not cause unacceptable adverse reactions, have only been able to delay, but not prevent, disease progression. As a potentially safer alternative or adjunct, we have been investigating the utility of mAbs targeting defined peptide–major histocompatibility complex (MHC) II complexes that are the ligands for disease-relevant $CD4^+$ T cells. Alleles within the MHC class II locus confer the greatest genetic risk for T1D, and activation of pathogenic $CD4^+$ T cells by antigen-presenting cells (APCs) expressing these ligands is central to disease etiology. Consequently, selective disruption of these critical interactions should arrest autoimmunity without causing global immunosuppression. Here, we review studies using an mAb targeting a key pathogenic epitope from insulin to treat a spontaneous mouse model of T1D and discuss the translational potential of therapies based on this approach.

T1D

Most individuals, but not all, with a diagnosis of type 1 diabetes have the immune-mediated form of the disease (type 1A), which results from T cell-mediated β cell destruction (Eisenbarth, 2010). The resulting severe insulin deficiency causes persistent hyperglycemia and a life-long dependency on an exogenous source of the hormone (reviewed in Atkinson et al., 2014). T1D has a major genetic component, the strongest risk factors deriving from genes within the MHC, and regulatory regions within the insulin gene itself. Notably, polymorphic variants of genes encoding MHC class II molecules confer ∼40%–60% of genetic susceptibility (Atkinson et al., 2014), highlighting the key role that $CD4^+$ T cells likely play in the pathogenesis of the disease. Prospective analysis of individuals carrying high-risk genes indicates that the onset of clinical T1D is typically preceded by the appearance of autoantibodies targeting islet cell antigens (ICAs), which can persist for years, or even decades, prior to overt dysglycemia (Atkinson et al., 2014). At present, the environmental factor(s) that trigger this persistent islet autoimmunity remain uncertain, but it is clear that they must foster a breech in tolerance that allows autoreactive T cells to acquire a pathogenic phenotype. Autoantibodies targeting ICAs such as pre(pro)insulin (IAA), GAD65, IA-2, and ZnT8 are widely used clinically, both for confirming a diagnosis of T1D and as inclusion criteria for prevention trials. However, only IAA positivity shows a significant correlation with the age of onset of clinical T1D in humans (Steck et al., 2011). This suggests that insulin is a particularly important target of islet autoimmunity and thus a rational target for antigen-specific immunotherapy (ASI) to suppress the unwanted responses.

Immunotherapy for T1D

Although clinical trials designed to arrest or reverse disease progression by immune intervention have been ongoing since the 1980s, despite many promising results, an effective treatment suitable for widespread clinical use is still lacking (reviewed in Atkinson et al., 2019). This disappointing situation likely reflects the fact, which in part has emerged from the results of the trials, that we still do not fully understand the pathogenesis of the disease in humans or the full impact on outcomes of individual differences in demographic and environmental factors. Broadly speaking, most immune intervention trials in T1D have adopted one of two alternative strategies, specifically, testing drugs that either target one or more key immune cell subsets implicated in disease etiology (Atkinson et al., 2019) or that are designed to modulate the immune response to a particular antigen expressed by pancreatic β cells (Roep et al., 2019). Each approach has both advantages and disadvantages and has shown some promise of providing clinical benefit, although there is a growing consensus that a single agent therapy is

Table 1 Key advantages and disadvantages of mAbs for immunotherapy in T1D.

Approach	Advantages	Disadvantages	Examples
Global modulation	HLA agnostic; mechanism understood	Potential for adverse events from global immune suppression; potentially genotype restricted	Teplizumab; Rituximab
Antigen-specific	Low likelihood of adverse side-effects; multiple formats possible	Restricted to defined HLAs; mechanism not yet understood	mAb287

probably unlikely to be successful, and that ultimately combinatorial approaches that target multiple aspects of immune and β cell biology will likely be required (Atkinson et al., 2019; Roep et al., 2019). To date, mAbs have only been used for global immunomodulation. Some potential advantages and disadvantages of their use for ASI are shown in Table 1.

Insulin autoimmunity in mice

The non-obese diabetic (NOD) mouse is the most widely studied spontaneous model of human T1D and shares many of the same genetic risk factors. Initial studies of islet autoimmunity revealed that insulin is a major target of pathogenic $CD4^+$ T cells in these animals and that a peptide comprising residues 9–23 of the B chain (B:9–23) contains at least one key epitope (Wegmann et al., 1994). Subsequently, a seminal study by Eisenbarth and colleagues showed that NOD mice that only express an *Ins2* variant, in which the native tyrosine at position 16 in the B chain is replaced by alanine (which disrupts the B:9–23 epitope without impacting insulin's biological activity), are completely protected from spontaneous disease (Nakayama et al., 2005), suggesting that T cells targeting this epitope may be critical for initiating disease in these animals.

The NOD mouse expresses a single MHC class II molecule, I-A^{g7}. Like HLA-DRB1*03:02, which is strongly associated with human T1D, I-A^{g7} lacks a canonical Asp residue at position 57 of the β chain. This residue normally forms a salt bridge with the conserved Arg at position 76 of the α chain to constrain the P9 peptide-binding pocket. Substitution of $Asp^{\beta57}$ with Ser (I-A^{g7}) or Ala (DQ8) 'loosens' the P9 antigen-binding pocket and confers a strong preference for binding peptides with acidic residues at this position (Suri et al., 2002). Conversely, binding of peptides with a basic residue at P9 to I-A^{g7} is energetically unfavorable. However, this creates a potential conundrum, as anchoring B:9–23 via Glu_{21} would place the critical Tyr_{16} residue in the P4 pocket of I-A^{g7}, whereas the mutational studies suggest that it directly interacts with the T cell receptors (TCRs) on islet-infiltrating T cells and likely occupies either the P3 or P5 position (reviewed in Nakayama et al., 2005). Two groups have attempted to resolve this issue, albeit with apparently conflicting results. Thus, using a series of truncated peptides, Unanue and colleagues concluded that pathogenic T cell clones recognize B:9–23 bound with either Gly_{20} or Glu_{21} in the P9 pocket (Levisetti et al., 2007). In contrast, using two distinct methods to 'fix' or 'trap' the peptide in defined binding registers, Kappler and colleagues reported that, unexpectedly, the same T cells all recognize B:9–23 in the 'third' binding register (InsB:R3), which places the highly unfavorable Arg_{22} residue in P9 (Stadinski et al., 2010). More recently, the same group has solved the structures of several tri-molecular complexes containing I-A^{g7}, B:9–23, and 'representative' diabetogenic TCRs by X-ray crystallography, obtaining results that are both consistent with their earlier mutational analyses and suggestive of the potential involvement of peptide splicing as a mechanism to resolve the apparent paradox of the P9 residue (Wang et al., 2019).

Targeting the I-A^{g7}/InsB:R3 complex in NOD mice is an effective immunotherapy

Several lines of evidence suggest that selective targeting of the I-A^{g7}/InsB:R3 complex is effective in altering disease progression in the NOD preclinical model. First, immunization of young female animals with recombinant I-A^{g7}/InsB:R3 complexes in the absence of adjuvant delayed disease, while immunization with control I-A^{g7}/HEL complexes did not (Zhang et al., 2011). Second, repetitive administration of mAb287, a 'TCR mimetic' mAb that was generated by immunization with I-A^{g7}/InsB:R3 complexes but not a matched IgG1 isotype control, protected approximately half of the treated mice from developing T1D (Zhang et al., 2014). Importantly, mAb shows no cross-reactivity with native insulin, the free peptide, B:9–23 presented in other registers by I-A^{g7}, or I-A^{g7} that is either empty or occupied by an irrelevant peptide. Indeed, structural analysis of mAb287–I-A^{g7}/InsB:R3 complexes confirms that the antibody mimics TCR binding, as there are major contacts both with exposed residues of the bound peptide and adjacent regions of I-A^{g7} (unpublished data), explaining its high specificity for the complex. This exquisite specificity, along with the fact that despite targeting an insulin-derived peptide mAb287 does not disturb hormonal activity, makes it a highly safe intervention. Moreover, mAb287 presumably acts by selectively targeting only those APCs that are presenting InsB:R3. As the antigen likely originates *in vivo* mostly from dead or diseased β cells, these APCs are also likely to be simultaneously presenting pathogenic complexes from other ICAs. If mAb287 acts not simply as a blocking agent but also influences the function, trafficking, or survival of the targeted APCs (Figure 1A), this would explain its ability to suppress responses to other ICAs *in vivo* but not *in vitro* and efficacy even at late stages of the disease when

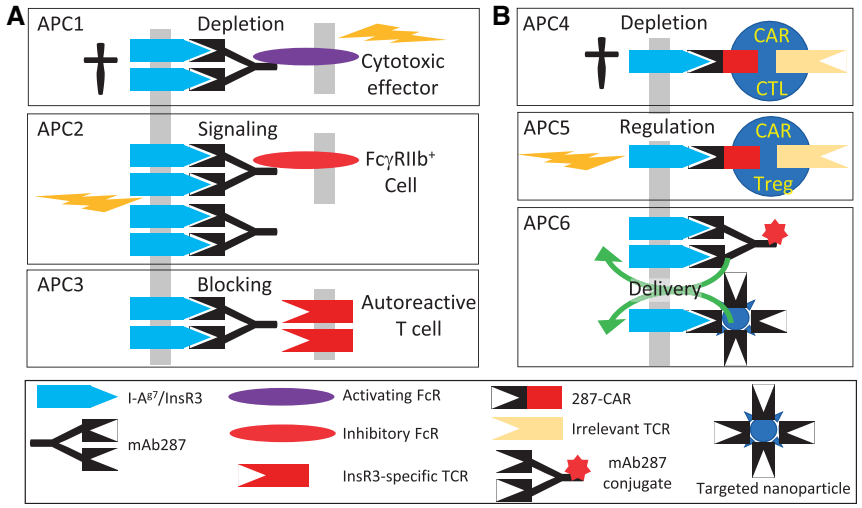

Figure 1 Potential modes of action and applications of mAb287 and related antibodies targeting pMHCs. (**A**) Binding of mAb287 to APCs presenting I-A^{g7}/InsR3 complexes may lead to the activation of cytotoxic effector cells and APC depletion (APC1), the transduction of a signal to the APC that either alters its function or induces apoptosis (APC2), and the blockade of InsR3-specific T cells (APC3). (**B**) Derivatives of mAb287 can be used to generate CARs to re-target cytotoxic (APC4) or regulatory (APC5) T cells or to target conjugates (Birrer et al., 2019) or nanoparticles (Zhuang et al., 2019) to the APC for drug delivery (APC6). Panel **A** is modified from Beers et al. (2016).

extensive epitope spreading has already occurred (Zhang et al., 2014).

A limitation of the mAb287 monotherapy described above is that it does not appear to induce a durable tolerance, necessitating frequent injections due to the limited half-life of the antibody and the continuous acquisition of autoantigens by newly recruited APCs. A potential solution to this limitation is to use a cell-based therapy. One possibility would be to engineer hematopoietic stem cells to express mAb287 after B cell differentiation, while a second would be to create a chimeric antigen receptor (CAR) that could be used to redirect other lymphocytes. At present, the precise mechanism of action of mAb287 is unclear, but one hypothesis is that it targets the relevant APCs for selective elimination. In this scenario, a cytotoxic T cell with the same specificity could be equally effective and have the added advantage of an extended lifespan *in vivo*. This can be achieved using a CAR. As expected, engineered CD8$^+$ T cells expressing a mAb287-CAR selectively killed APCs expressing I-A^{g7}/InsR3 *in vitro*. Moreover, after adoptive transfer to young NOD mice, the mAb287-CAR T cells trafficked to the sites where their target cells are located and were able to cause a significant delay in the onset of T1D in the treated animals (Zhang et al., 2019). However, under the conditions used, the mAb287-CAR T cells showed only limited expansion and longevity and thus the final incidence of T1D was not different in animals treated with mAb287-CAR T cells from those treated with an irrelevant control (Zhang et al., 2019). Thus, although promising, further optimization of the protocol is required.

Targeting other pathogenic peptide–MHC complexes in autoimmunity

To our knowledge, at present, mAb287 is the only 'TCR mimetic' antibody that has been shown to prevent onset of a spontaneous autoimmune disease. However, related antibodies targeting peptide–MHC complexes (pMHCs) implicated in the pathogenesis of multiple autoimmune conditions including T1D, multiple sclerosis, rheumatoid arthritis, and celiac disease have also been described (reviewed in Hoydahl et al., 2019). Indeed, this approach was first reported to prevent autoimmunity in 1991 when Aharoni et al. (1991) used it to inhibit induction of experimental autoimmune encephalomyelitis in H-2^s mice. However, studies since then have used the agents primarily for analysis of *in vivo* epitope formation, rather than for therapeutic purposes, and have otherwise been limited to confirming the ability of the antibodies to prevent activation of antigen-specific T cells in response to immunogens or pathogens.

Comparison of mAb287 with other forms of ASI

Unlike mAb287 therapy, most previous studies of ASI in T1D have involved immunization with the free antigen in either protein, peptide, or cDNA format (Roep et al., 2019). The goal of such studies has generally been to restore tolerance by preferentially inducing or expanding populations of antigen-specific T cells with regulatory phenotypes. A number of trials have been conducted, and critically, all have proved safe, with several providing some preliminary evidence of clinical benefit in at least some individuals (Roep et al., 2019). The main advantages of this 'conventional' approach over an anti-pMHC-based treatment such as mAb287 are that some antigen formats (peptides and cDNA) are much simpler and cheaper to manufacture under Good Manufacturing Practice (GMP) conditions than an antibody and, in the case of proteins and cDNA, that the agent can be used in any individual irrespective of their HLA genotype. Conversely, potential limitations of 'conventional' ASI include its likely dependence upon the presence of a sufficient pool of naïve T cells of the targeted specificities to generate an effective tolerogenic response and propensity for effector T cells to become refractory to regulation in some subjects (Buckner and Nepom, 2016). In contrast, the main advantage of anti-pMHC-based therapies is that they directly target the specific subsets of APCs that are actively involved in promoting a pathogenic

response, and thus should be unaffected by the factors described above that could frustrate the 'conventional' approach. However, this must be balanced against the higher cost of generating the antibody and the need to develop a panel of reagents to accommodate individuals with different HLA genotypes.

Insulin autoimmunity in humans

As discussed above, insulin is an early target of autoantibodies in humans. Numerous studies have shown that it is also a major target of both $CD4^+$ and $CD8^+$ T cells in patients with T1D, with potentially pathologic cells restricted to multiple MHC molecules and targeting epitopes throughout the preproinsulin molecule expanded in the circulation of many individuals and also detectable within insulitic lesions (Coppieters et al., 2012; Atkinson et al., 2014; Michels et al., 2017). T cells targeting similar pMHCs are also detectable in the circulation of healthy subjects, although in this case they more typically exhibit a naïve or anti-inflammatory phenotype (Nakayama et al., 2015). Approximately 50% of patients with T1D express at least one copy of HLA-DQ8, and close to onset pro-inflammatory $CD4^+$ T cells specific for B:9–23 can be detected in the blood of many of these individuals (Nakayama et al., 2015). Moreover, some of these T cells target InsB:R3 (Yang et al., 2014; Wang et al., 2019), suggesting that DQ8/InsB:R3 complexes (and by extension the cognate antigens of other potentially pathogenic $CD4^+$ T cells) could also be legitimate targets of ASI in humans.

Potential uses of anti-pMHC antibodies in the clinic

The therapeutic use of mAbs is now well established and growing at a significant rate. Collectively, drug companies are currently sponsoring trials of >500 new mAbs for treating a wide variety of diseases, with more drugs in the pipeline (Kaplon and Reichert, 2019). In many cases, mAbs are used either as surrogate high-affinity ligands to directly trigger a desired cellular response or, conversely, to block an undesirable receptor–ligand interaction (Beers et al., 2016; Figure 1A). However, mAbs are also used therapeutically to enhance the selective delivery of another drug to a particular tissue. In its simplest form, this is achieved by linking the payload (such as a cytotoxic drug or radionucleotide) directly to the targeting antibody, typically via cysteine or lysine residues, to form an antibody–drug conjugate (Birrer et al., 2019). Alternatively, antibody derivatives can be used to target nanocarriers such as liposomes, polymers, or virus-like particles that encapsulate molecules including lipophilic drugs and synthetic RNAs or DNAs that are unstable in the circulation (Zhuang et al., 2019). Antibodies targeting pMHCs could be used in both of these modalities, e.g. to enhance deletion of a particular APC subset or to deliver an anti-inflammatory compound (such as IL-10) to modulate the autoimmune response (Figure 1B). mAbs are also increasingly being used clinically as targeting elements for CARs. This application was pioneered in the field of cancer immunotherapy and is having a transformative effect on modern clinical care in this speciality. However, the same approach can be directly translated to treating autoimmunity (Maldini et al., 2018). As discussed above, our preliminary studies suggest that cytotoxic CARs targeting human pMHCs may also have therapeutic utility in autoimmunity. Moreover, whereas the primary goal of cancer immunotherapy is to eliminate the tumor, the 'holy-grail' of autoimmune research is to restore tolerance. Thus, a potential alternative strategy is to use pMHC-directed CARs to re-target regulatory T cells (Tregs; Figure 1B), given that endogenous islet-specific Tregs in T1D patients may be defective in quality or quantity (Bluestone et al., 2015). Since it is technically difficult to identify and expand rare endogenous antigen-specific Tregs for therapeutic purposes, adoptive transfer of re-directed polyclonal Tregs may overcome many of the existing barriers. It is also important to note that, as discussed above, the therapeutic utility of mAbs targeting pMHCs is not limited to T1D but in principle can be applied to any other condition for which a pathogenic complex is known.

Future directions

To date, the use of mAbs to treat T1D has mainly focused upon drugs such as teplizumab and rituximab that deplete or modulate major populations of immune cells implicated in pathogenesis (Ludvigsson, 2016; Table 1). These agents have shown considerable promise but have an inherent potential to cause immune suppression that limits the doses that can be safely used. The data discussed above suggest that antibodies targeting pathogenic pMHCs might be a safe alternative, although many key variables will need to be defined before they are ready for clinical use. There is a growing appreciation that T1D is quite heterogeneous and likely has multiple endotypes (Battaglia et al., 2020). This may explain why only a subset of subjects responded to teplizumab and rituximab in previous clinical trials and highlights the need for greater mechanistic insight into the precise *in vivo* mode(s) of action of mAbs targeting pMHCs. For example, does the mAb simply block the interaction between the APC and T cell or alter the function, trafficking, or survival of the target cell, and if so, which? Does the mAb target all APCs equally or a critical subset? Does the mAb act autonomously or dependently upon another cell population, and if so, which? Can the efficacy be enhanced by simultaneously targeting multiple pathogenic pMHCs, e.g. in the form of a bi-specific antibody or mAb cocktail? Answers to these questions are actively being sought, and their resolution will likely impact future trial design.

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