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Review article

T cell response to FVIII

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ABSTRACT

Several lines of evidence indicate that the immune response to Factor VIII (FVIII) in patients with hemophilia A is T cell-dependent. This review highlights the link between the epitope specificity of FVIII-specific T cells and their potential roles in different categories of patients. FVIII-specific T cells able to recognize wild-type (i.e. therapeutic) FVIII but not the mutated self FVIII of hemophilia patients have been identified in patients with mild/moderate hemophilia carrying some point mutations. Such T cells likely contribute to the higher frequency of neutralizing anti-FVIII antibodies (inhibitors) development in these patients. In contrast, as yet no T cells have been identified that can differentiate between FVIII molecules with non-hemophilia-causing single amino acid variants encoded by non-synonymous single-nucleotide polymorphisms in the F8 gene. Other mechanisms are therefore still to be identified that will explain the clinically noted differences in the incidence of inhibitor development between patients of different races who are known to have differences at these sites. Beside information about the mechanism of inhibitor development, the analysis of FVIII-specific T cells has provided tools to develop novel diagnostic and therapeutic approaches, such as the generation of FVIII-specific regulatory T cells that may be useful in preventing or suppressing the immune response to FVIII.

Contents

1. Introduction

Patients with severe hemophilia A (FVIII activity less than 1%) who are treated by the administration of recombinant or plasma-derived FVIII concentrates develop FVIII inhibitory antibodies in about 20–40% of cases [1]. Several lines of evidence indicate that the immune response to FVIII is T cell-dependent. First, the observation that a large proportion of anti-FVIII antibodies belong to the IgG4 subclass [2] pinpoints a role for T cells in the development of the humoral response to FVIII since isotype switching is T cell-dependent. Second, hypermutations are consistently detected in the genes coding for the variable part of anti-FVIII antibodies obtained either by immortalization of peripheral blood lymphocytes of patients with inhibitor [3] or by phage display technology [4]. This indicates that B cells producing anti-FVIII antibodies undergo affinity maturation processes, which also require specific T cell help [5]. Lastly, in patients with an established humoral response to FVIII, HIV infection leads to the disappearance of FVIII inhibitor when T cell counts decline, demonstrating the requirement for T cells in this process [6]. The objective of this review is

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to highlight the epitope specificity and the potential role of FVIII-specific T cells that have been identified in patients with hemophilia A and inhibitor.

2. FVIII-specific T cells in control subjects and in patients with hemophilia A and inhibitor

FVIII-specific T cells were first identified in the peripheral blood of hemophilia A patients with inhibitor using T cell proliferation assays with native FVIII [7]. The antigenic determinants (epitopes) recognized by such T cells were studied using synthetic peptides covering the entire FVIII molecule [8]. Epitopes were mapped in all domains of the FVIII molecule.

Surprisingly, T cells proliferating in response to FVIII peptides have also been identified in hemophilia A patients without inhibitor as well as in healthy individuals. In these reports only minor differences were identified between FVIII-specific T cells from healthy individuals and those from hemophilia A patients with or without inhibitor [8–11]. This lack of clear-cut difference at the T cell level has raised questions about the role of FVIII-specific T cells in the response to FVIII. It may be the case that FVIII-specific T cells are unnecessary for the development of an immune response to FVIII, although this seems unlikely given that in patients with an established humoral response to FVIII, HIV infection leads to the disappearance of FVIII inhibitor when T cell counts decline [6]. Alternatively, qualitative differences in the phenotype of FVIII-specific T cells, such as the profile of interleukin production, may sustain tolerance or the induction of an immune response to FVIII [12,13].

Interestingly, in addition to FVIII-specific T cells, antibodies to FVIII have also been isolated from control subjects and from hemophilia A patients without clinically-relevant inhibitor [14–16]. The paradoxical presence of anti-FVIII antibodies in subjects with normal FVIII levels has only recently been explained by the observation that the affinity of these antibodies is much lower than that of inhibitory antibodies [17].

3. FVIII-specific T cells in patients with mild/moderate hemophilia A and inhibitor

In contrast to patients with severe hemophilia A, the development of inhibitor is rare in mild/moderate hemophilia A patients (FVIII: 1–40%) [18]. Nevertheless, mild/moderate patients carrying certain mutations present an incidence of inhibitor formation comparable to that of patients with severe hemophilia A. Such mutations have been located in the amino-terminal part of the C2 domain of FVIII, the carboxy-terminal part of the C1 domain, and the amino-terminal region of the A2 domain [19].

Analysis of polyclonal and monoclonal anti-FVIII antibodies derived from patients with mild/moderate hemophilia A and inhibitor demonstrated that certain antibodies exclusively recognized epitope(s) present on wild-type FVIII but not on mutated counterparts carrying the Arg593Cys or Arg2150His substitutions [20–23]. The B lymphocytes of such patients were therefore able to make the distinction between self (mutated) and exogenous (normal) FVIII. These observations raised the question of whether FVIII-specific T cells from patients with mutated FVIII could also recognize epitopes on normal exogenous FVIII that were absent on the patients' own mutated FVIII. Two techniques were exploited to answer this question.

The first approach allowed the expansion and characterization of T cells present in the blood irrespective of their epitope specificity. Using this method, the T cell response to FVIII was studied in a mild hemophilia A patient carrying an Arg2150His substitution in the C1 domain who presented with a high-titer inhibitor toward normal but not self-FVIII. T cell lines from this patient were expanded and cloned by stimulation with FVIII presented by autologous FVIII-specific B cell lines. These FVIII-specific T cells recognized a peptide encompassing residue Arg2150 of FVIII, the residue mutated in the patient's FVIII gene, and did not recognize recombinant FVIII carrying the substitution Arg2150His. This demonstrated that the C1 domain of wild-type FVIII contains T cell epitopes that are absent in FVIII carrying the mutation Arg2150His [24]. This mutation likely alters the ability of T cells to recognize the mutated FVIII peptide associated with MHC molecules, since immunopurified MHC class II molecules were found to bind to the mutated peptide nearly as effectively as the native peptide.

In a separate approach, FVIII-specific T cells from patients with mutated FVIII were amplified using peptides derived from normal FVIII that corresponded to the region where the mutation was located in the patients' own FVIII gene. This technique can also be used in tetramer guided epitope mapping, where the specific T cells involved can be identified after labeling with a tetramer of MHC class II molecules loaded with the relevant peptide. However, although this technique has proven very efficient at identifying T cells of a predefined specificity, it cannot provide information about T cells that recognize epitopes in other regions. Using this approach, the T cell response of a subject with mild hemophilia A with the missense genotype was characterized for 1 year following his initial inhibitor response [25]. His T cells were stimulated and expanded with pools of peptides covering the C2 domain.

With this method, a T cell epitope sequence containing the 2201 residue was identified. Interestingly, peptides containing the native FVIII Ala2201 were recognized by the patient's CD4 T cells collected up to 1 year after inhibitor development, whereas the mutated Pro2201 peptide was recognized only around the time of the initial peak response. These results were confirmed by the isolation of T cell clones that both proliferated in response to peptides containing Ala2201 and recognized the Ala2201 peptide bound to MHC-class II tetramers. Unexpectedly, no T cell clones recognized the hemophilic (Pro2201) peptides. These results suggest that the epitope specificity of the FVIII-specific T cells evolved over time in this patient, leading to a loss of recognition of the self-epitope. However, the relevance of T cell recognition of the self-peptide in vivo remains unclear because binding of the mutated peptide to the patient's MHC class II molecules was about 20-fold weaker than the binding of the wild-type peptide [25].

T cell clones recognizing FVIII peptides involving residue 2201 have also been derived by tetramer guided epitope mapping from another patient with the FVIII-Ala2201Pro genotype and a non-clinically-significant inhibitor response. Several clones recognized peptides containing the Ala2201 residue. In contrast to the above case, these clones were also activated by peptides containing Pro2201 [26], however the concentration of mutated peptide required to activate the T cells was between 30- and more than 100-fold higher than that of the native peptide, again raising the question of whether such T cells can actually be activated by the autologous mutated FVIII molecule in vivo [26].

FVIII-specific T cell clones recognizing peptides involving another hemophilic missense site, Arg593Cys, have also been identified in two unrelated subjects with mild hemophilia A [27]. Unexpectedly, peptides containing both the wild-type Arg593 residue and the hemophilic Cys593 residue elicited similar levels of proliferation of the T cell clones. These observations do not fit with the concept that presentation of wild-type FVIII epitopes rather than the mutated patient FVIII increases inhibitor risk by activating T cells that do not recognize self-FVIII. However, in tetramer guided epitope mapping, CD4+ T cells from patients were stained by tetramers loaded with wild-type and not mutated peptides, suggesting a lower-avidity interaction of T cells with the tetramers and thus possibly with antigen-presenting cells [27].

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