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Biologics and postbiologics: novel immunotherapeutics for the induction and maintenance of remission

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Rapid growth in the development of new tools and advances in molecular immunology has led to a class of drugs called biologics. While effective, these therapies are costly, typically require continuous administration and often have serious side effects. This review focuses on current therapies and the need for a new class of therapies. A novel class of drugs able to induce and maintain tolerance will be paralleled with the notion that combination therapy appropriately tailored to patient subpopulations may hold the promise of less costly and more effective therapy.

Introduction

A very rapid technical evolution is dramatically reshaping the arsenal of tools available for the treatment of rheumatoid arthritis. These new tools capitalize on recent gains in molecular immunology [1]. This recently spawned family of drugs is often bundled under the designation of 'biologic' or 'immunotherapies' [2–5]. In reality, a single designation does not do justice to the diversity within this group of drugs. Such diversity reflects the complexity and multiplicity of the immune pathways which contribute to disease pathogenesis [6–9]. This review will take into account these functional aspects and underscore also the complexity and uniqueness of the itinerary needed to develop these drugs from discovery to product.

We will first provide an outlook of the challenges the development of these drugs face and list some of the many drugs which are being developed. Then, we will review clinical experience with therapies currently available on the market. We will finally provide a short account of our own experience in the area of development of the induction and maintenance of tolerance as an approach to immunotherapy.

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Development of novel immunotherapies: the challenges of translational research

As with any new therapy, the development of an immunotherapy is composed of a series of steps, which are summarized in Fig. 1. This itinerary is, unfortunately, disconnected, leading to the demise of promising programs because of the lack of a coherent development plan, which should take into account challenges specific to the field. In the immunotherapy of autoimmune diseases, there are some specific challenges that cannot be solved with conventional approaches and that often hinder development: (i) lead identification and validation; (ii) CMC, toxicity and proof of concept in non-human species; (iii) clinical development, in particular outcomes and biomarkers.

Lead identification and validation

An initial hypothesis will identify a lead compound or approach for intervention. There are different possible approaches to identify a lead. In most cases, a single molecule or pathway is targeted by developing, for instance, a competitive inhibitor of the natural receptor or a soluble mimic of the receptor to interfere with the action of an undesirable protein or to reduce its circulating amount. This strategy is a traditional one. It is highly focused and has been proven effective, leading to most of the biologics currently in use. Evidence keeps accumulating, however, regarding the multiplicity of pathogenic elements which interlace in sustaining the autoimmune disease process. Clusters of immune

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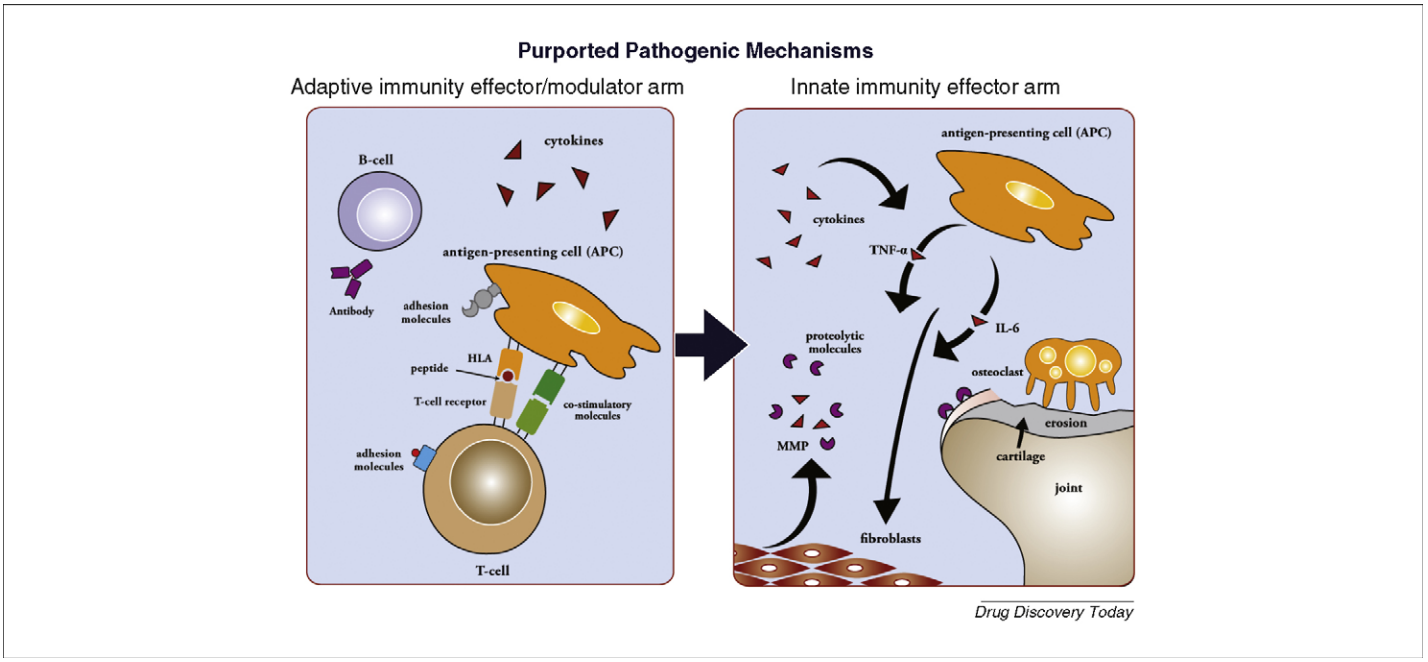

FIGURE 1

Purported pathogenic mechanisms. Left side depicts mechanisms of the adaptive immune response, while the right side depicts mechanisms of the innate immune response.

functions rather than individual elements should perhaps be also targeted by innovative approaches. A list of possible targets and pathways is represented as Fig. 2.

Toxicity and proof of concept in non human species

Evaluation of toxicity, pharmacodynamics and lead validation in animal models is a cornerstone of the traditional drug development process. Accordingly, animal models have been employed to develop biologics currently used in the therapy of rheumatoid arthritis. In some instances, including our own experience [10], the development of novel immunotherapies has required an innovative and not yet standardized approach to animal studies. In general, the major conceptual difference is that some of the therapeutic targets are uniquely human and, therefore, proof of safety and efficacy are hampered by the lack of a corresponding animal model [11]. In the case of autoimmunity, this is particularly evident, because it is relatively easy to induce experimental arthritis using the same antigen, which can be then used for immunotherapy. This approach does not necessarily reflect the situation in humans, where the triggers for autoimmunity are unknown, most likely multiple and probably irrelevant by the time the disease has established itself. Similarly, therapies targeting pathways that have different pathogenic relevance across species are of little value and yet they may be imposed by the regulatory agencies for the lack of a better alternative.

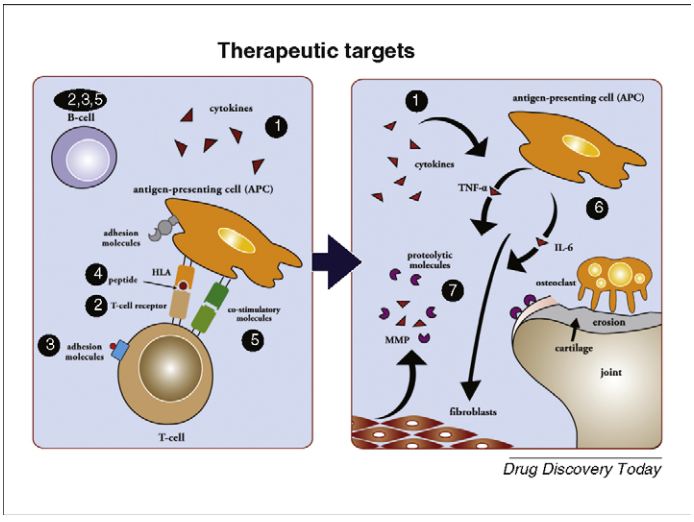

FIGURE 2

Therapeutic targets and pathways are depicted. (1) Anticytokine therapies. (2) Therapies directed toward the T or B cell receptor. These include depleting or blocking antibodies but also tolerization approaches. (3) Approaches interfering with adhesion and migration of the immune cells. (4) Antigen specific tolerization. (5) Therapies interfering with pathways related to the modulation of the intensity and quality of immune responses. These pathways rely on interactions among costimulatory molecules. (6) Therapies interfering with intracelular signaling. (7) Inhibitors of the final mediators of damage (i.e. proteolysis, MMPs).

Clinical development, in particular, outcomes and biomarkers

In some instances the wide array of technologies and knowledge available thanks to molecular immunology have not been exploited yet. Consequently, the information related to the mechanism of action of immunotherapeutics has not been matched with clinical outcome. This is also the consequence of the lack of appropriately powered and controlled studies designed to address mechanistic questions related to the effects of immunotherapy on the pathogenesis of autoimmunity. A typical example is provided by the inconsistencies and incomplete knowledge in the area of the effects of anti-TNF agents on adaptive immunity [4,5,12–15]. The speed of growth of immunotherapy will be hampered as long as the opportunity of using immunology to identify and even predict susceptibility to immunotherapy is missed.

Strategic therapeutic needs

An important aspect driving immunotherapy is the shift from conservative treatment, often seen as management of disease symptoms in the middle or advanced stages of the disease course, to early aggressive intervention that seeks to induce remission and, in large part, prevent debilitating damage before onset (such as joint damage in rheumatoid arthritis (RA) [16–18].

In RA, a combination therapeutic approach with the use of disease modifying antirheumatics (DMARDs) and biologics has become standard practice [19–21]. The main challenge of this tactic, which is indeed the challenge of biological monotherapy, is the maintenance of low disease activity and remission, once achieved. Although follow-up data to the study conducted by Allaart *et al.* indicate a lower incidence of relapse with DMARDs plus biologics or prednisone, maintenance of clinical and immunologic disease control is inconsistent and unachievable for the majority of patients [2,22–24].

Recent data from the BesT trial [25], however, show that in a minority of patients treated with a combination of DMARDs, including methotrexate and a TNF blocker, remission was maintained upon discontinuation of the therapy. The proportion of patients who maintained remission was low and the follow-up was still short at the time of writing. A prudent interpretation for these data is that the therapeutic approaches currently available have the ability to induce, but not maintain, remission. The next generation of immune therapy drugs will face the challenge of matching the efficacy of currently available biologics, while also reducing costs and side effects. This challenge can probably be met only by evolving the target focus from nonspecific to disease-related pathogenic mechanisms.

One possible avenue will be related to targeting, in an effective way, mechanisms of innate immunity more at the cellular level. Inhibition/modulation of innate immunity has, however, inherent risks linked to the possibility of impairing crucial functions of the first line of immune defence. Hence, it is probable that the field will evolve into integrating approaches targeted to adaptive immunity with the current therapeutic strategies.

There are multiple ways to manipulate adaptive immunity therapeutically in a specific fashion. Efforts aimed at interfering with B or T cell populations on the basis of interference with membrane receptors (such as CD20, CTLA-4 or CD3) have in several cases already reached the market [26,19,20]. Their virtues and limitations have been discussed above. The remainder of this review will rather focus on current efforts, including our own, in the area of modulation of antigen-specific T cells.

Modulating T cells in autoimmune diseases

An everlasting and, frankly, stale debate regarding which cell population is responsible for the etiopathogenesis of rheumatoid arthritis is being reshaped by the notion that all the components of the immune system contribute to the complexity and diversity of the pathogenic pathways. T cells are, however, arguably one of the most sophisticated components of the adaptive arm of the immune system. They perform a multiplicity of functions, ranging from effector to suppresssor to modulator. The ability to affect specifically their function for therapeutic purposes is a prized, but still elusive, target.

T cell crosstalk and T cell vaccination

T cell vaccination conceptually relies on the recognized ability of CD4 T cells to present antigens to other T cells. Even though this is not the most effective mechanism of T cell activation, it has proven useful in downregulating pathogenic T cell responses.

In animal models of autoimmunity, T cell vaccination is an effective treatment, not only of experimental autoimmune encephalomyelitis (EAE), but also of experimental diabetes mellitus, myasthenia gravis and arthritis, for example, collagen-induced arthritis and adjuvant-induced arthritis [27,28].

Studies in animal models have shown that at least two types of T cells are induced by T cell vaccination: anti-idiotypic and anti-ergotypic T cells [29]. The anti-idiotypic response is a specific T cell response directed against the autoimmune T cells targeted in a vaccine. Anti-idiotypic T cells respond to peptides derived mainly from the CD3 region of the TCR of autoimmune T cells. The anti-idiotypic response consists of a CD4 T cell response against TCR peptides presented by autoimmune T cells in the context of class II MHC, followed by a CD8 T cell response against TCR peptides presented by class I MHC (HLA–E in humans, Qa-1 in mice) on autoimmune T cells [29,30].

This mechanism, which is, however, specific for the autoimmune T cells driving the disease, does not account for all the effects of T cell vaccination observed in animal models. By the late 1980s, investigators had found that T cell vaccination is successful only if T cells are activated *in vitro* before injection; resting T cells are not effective. A second observation was that attenuated activated T cells with specificities other than those of autoimmune T cells are also effective in EAE [31].

The main challenge for translating this approach into human therapy is the lack of adequate procedures to control and reproduce this mechanism in humans [32], particularly in a context in which large-scale *ex vivo* manipulation is needed.

More promising, *albeit* not yet proven with respect to efficacy, is the strategy focusing directly on administration of peptides derived from the T cell receptor to the patients [29,33,34]. Vaccination to TCR-derived peptides can be considered in itself a form of epitope-specific immunotherapy.

Epitope-specific immune therapy

Tolerization to an antigen that is the inciting agent of an autoimmune process is a potentially attractive therapeutic option as the approach would, at least theoretically, be very specific and therefore prone to higher efficacy and fewer side effects. This concept has proven to be highly effective in the treatment of animal models of autoimmune disease in which the inciting agent is the same as the tolerogen. Following the traditional procedures of product development typical of the pharma industry, a wave of attempts to translate this approach in humans occurred approximately a decade ago, spawning initial enthusiasm and subsequent disappointment when the initially promising data were not confirmed in subsequent studies.

Examples of this approach were the testing of tolerization to oral type II collagen and to glycoprotein-39 epitopes in RA clinical trials [35–39]. The lack of complete success is probably the consequence of several hurdles that could not be entirely taken into account at the time. Among such hurdles are differences in protein glycosylation owing to the ongoing inflammatory process in RA

and individual variations in processing whole proteins, which may influence T cell recognition of putative autoantigens, as has been demonstrated for type II collagen [37–42]. Other problems may relate to the heterogeneity of the patient populations and the relative relevance that the purported triggering agent may have. These problems compounded may, in turn, affect the efficacy of a therapeutic approach on the basis of tolerization to proteins considered to be putative triggers of the disease.

Refining the target of epitope-specific immune therapy

The concept of modulating bystander immune pathways applies to disease pathogenesis. In RA, it is hypothesized that one or more antigens induce inflammation and tissue damage. Instead of a physiologic downregulation, the inflammation amplifies nonspecifically, involving epitopes which were not originally the target of the reaction. Owing to this mechanism of epitope spreading, the process can continue in a self-perpetuating loop and the initial triggers may be of little relevance to the process. Hence, it may be difficult to aim at an elusive antigen involved in triggering the disease for therapeutic purposes.

The first conceptual change to consider in immunotherapy may be that the search for the trigger of human autoimmune disease could be refocused on antigens participating in the mechanisms of amplification and control of inflammation. An approach of this type may be fundamental in overcoming one of the major limitations of epitope-specific therapy in humans, that is the fact that the triggers are elusive and possibly multiple. More importantly, this approach may target mechanisms that are relevant and central to the perpetuation and modulation of the autoimmune inflammation generated by both the innate and the adaptive arms of the immune system, thus increasing the chances for the treatment to be clinically relevant.

A translational itinerary of development for nontrigger-specific immunotherapy from animal models to human disease

Potential candidate antigens need to fulfill certain characteristics. In particular, candidate antigens should be present and possibly overexpressed at the site of inflammation. They should be immunologically relevant, thus triggering T cell responses and production of cytokines that contribute to modulating the inflammatory process locally and systemically. Self-antigens with these characteristics often have molecular mimics whose cross-recognition may be necessary to initiate or modulate the immune reactivity. Several families of antigens with these characteristics have been described [43–45]. Heat shock proteins (hsps) are among the most notable of such proteins. In recent years, considerable progress has been made in characterizing the central role of hsp in the modulation and amplification of inflammation, in both health and disease [29,45–48].

hsp are evolutionary, highly conserved proteins that are present in the cells of virtually all living organisms and play essential roles in cell function. Expression of hsp is upregulated during conditions of cellular stress, including infection and inflammation, and as such they are a readily available antigenic challenge (Fig. 3). Increased expression of endogenously produced hsp60 and hsp dnaJ has been documented at various sites of autoimmune inflammation [49–53].

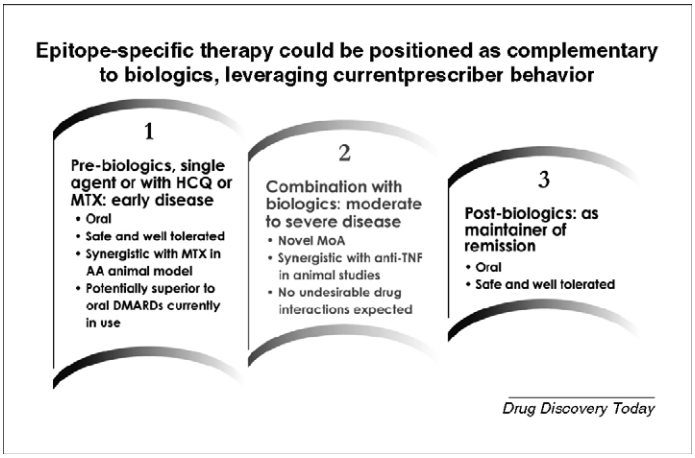

FIGURE 3

Challenges in novel immunotherapeutic development. This figure shows the translational medicine development pathway. MOA, mechanism of action.

As discussed, recognition of hsp is by default perceived as a danger signal by the immune system, thus triggering a proinflammatory response that involves both the adaptive and innate arms of the immune system. T cell proinflammatory responses to hsp are found in several autoimmune diseases, including RA, juvenile idiopathic arthritis (JIA), juvenile diabetes mellitus, multiple sclerosis and inflammatory bowel disease [47,48,54]. hsp are not, however, purely proinflammatory antigens. Years of research in disparate settings have shown that recognition of hsp often occurs in chronic inflammatory diseases and is often associated with a remitting form of such diseases. Therefore, responses to hsp in autoimmunity seem to entail a certain degree of contradiction. hsp offer another venue of epitope-specific immunotherapy to explore more thoroughly and initial therapeutic intervention strategies exhibit encouraging signs of efficacy and safety.

Animal models have been pivotal in the development of antigen-specific immunotherapies. Studies in antigen-induced disease models have demonstrated that the inducing antigen itself or related compounds, such as altered peptide ligands (APLs) [55,56], can be used for the down-modulation of disease producing immune effector T and B cells, even in established disease [57]. In addition, a variety of effector cells and mechanisms associated with this modulation, such as regulatory T cells and modulatory cytokines TGFβ and IL10, have been uncovered in animal models.

Both antigen-induced (e.g. proteoglycan and collagen-induced arthritis) and the so-called adjuvant-induced models are available for arthritis. The latter models are induced with mycobacteria in oil, or just synthetic or natural oily adjuvants. Besides the adjuvant models may resemble human disease, mycobacteria are known to produce arthritis also in humans, such as seen as a side effect of BCG immunotherapy in, for instance, bladder cancer [58]. They also have the advantage of not depending on specific antigens [59].

The immunomodulatory activities of mycobacterial hsp60 were discovered in the rat adjuvant-induced model of rheumatoid arthritis. Subsequent testing in both antigen- and adjuvant-induced models revealed the broad immuno-modulatory qualities of this molecule. Although the mechanisms involved are likely to be manifold given the complex biology of hsp, there is

accumulating evidence that conserved sequences of mycobacterial hsp60 are inducing regulatory T cells with the capacity to cross-recognize self-hsp60 overexpressed in stressed inflamed tissues. Such an antigen-independent bystander regulatory activity would explain why hsp are disease suppressive not only in adjuvant arthritis (AA), but also in other inflammatory conditions, such as antigen-induced arthritis, diabetes, atherosclerosis and several others [48].

Essential for the translation of the animal findings into human therapies is that the models used are relevant, which means that for the development of a therapeutic approach and toxicity testing in general, the test material must be pharmacologically active in the animal species used. For antigen-specific immunotherapies, the immune repertoire must be able to recognize the material. This can be tested relatively easily in both the model itself and in humans by *in vitro* screening.

Bridging the translational gap between model and disease in oral tolerance

Until now the success of translating effective oral tolerance based models into human therapies has remained limited. This is for several obvious reasons.

Regulatory agencies (and in many cases commercial investors) expect biological therapies to develop along the lines of classical drug therapies: one optimal dose to be effective in most of the patients with clinical improvement as the necessary endpoint. This, however, is difficult to achieve. Animal models can show optimal dosages relative easily, because the recipients are usually inbred and homozygous for crucial genetic elements, such as MHC. In patients dosing will depend on complex genetics and more variable environmental factors. Additionally, the disease in most cases will be less homogeneous; patients will be in different activity stages and have a different immune repertoire. Treatment efficacy will depend on the immunological effect that can be reached with the immune intervention. Therefore the critical endpoints need to be based on immunological monitoring. Such monitoring should reveal responders and nonresponders. As a consequence of this variable responsiveness, the more attractive antigenic compounds for therapies will be those that have shown their activities in multiple (inbred) strains of animals and preferably in multiple disease models. hsp are such examples of compounds that have shown broad activities in various disease models [48].

Epitope-specific immunotherapy in rheumatoid arthritis

We have attempted to translate the concepts outlined above into clinical practice. In preclinical work, we identified dnaJP1, a peptide derived from the hsp *E. coli* dnaJ, which shares homology with its human equivalent, as a candidate epitope [49,50,60]. dnaJP1 contains the five amino acid cassette QKRAA present on most of the HLA class II alleles associated with RA [61]. It has been designed in silica as a strong avidity pan HLA-DR binder. dnaJP1 is recognized by both PBMC and synovial fluid mononuclear cells in a majority of patients with active RA [49].

Next we explored in a Phase I/IIa study whether immune tolerization of T cells and APCs can be promoted through mucosal tolerization to dnaJP1. Fifteen patients were treated with open-label dnaJP1 for six months. Immunological analysis suggested an immune deviation. dnaJP1-specific T cells shifted from proinflammatory to a more regulatory function. This shift was accompanied by increased production of anti-inflammatory cytokines IL-10 and IL-4, whereas proinflammatory cytokines IL-2, TNFα and IFNγ decreased [62].

We subsequently investigated the clinical viability of this approach in a Phase II study. The double blind, placebo controlled, pilot Phase II trial was designed to test if immune tolerization to dnaJP1 translates into clinical benefit. To explore dnaJP1 as a first line agent, concomitant medications such as methotrexate and biologics were excluded. Patients were enrolled on the basis of dual clinical and immunological entry criteria, the latter consisting of documentation of a proinflammatory response *in vitro* to the peptide. One hundred and sixty patients with early RA were randomized and received orally 25 mg of dnaJP1 or placebo daily for six months, with monthly and follow-up clinical and laboratory assessments. T cell function measured immunological efficacy. American College of Rheumatology (ACR) 20 composite scores measured clinical efficacy. Immune deviation was achieved as a qualitative switch from proinflammatory to tolerogenic T cell responses to the peptide *in vitro*. Deviations in T cell responses were treatment-specific and pronounced in clinical responders. Notably, production of TNFα decreased while IL-10 production increased in the treatment group. Differences in ACR scores between the different groups became evident beginning at day 140 of treatment and diverged progressively at day 168 and follow-up, which may be indicative of the time needed for immune tolerization. Both trials demonstrated favorable safety profiles as no significant side effects were reported. The incidence of adverse events in the Phase II trial was comparable in the treatment and placebo groups. The results of the Phase II trial provide signals of efficacy encouraging enough for follow-up studies to further develop dnaJP1 as a novel therapeutic intervention.

The concept that the immune circuit we are targeting is not disease but rather inflammation specific is supported by data in other diseases in humans and in animal models of autoimmunity, such as EAE and IBD [63] The epitope-specific approach also has the potential to be combined with current therapeutics. In the animal model of RA (adjuvant-induced arthritis) we combined epitope-specific tolerization with anticytokine therapy, which led to full disease control. Moreover, the dose of anticytokine therapy

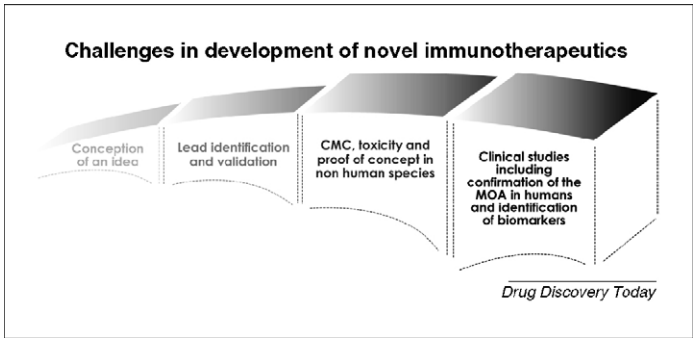

FIGURE 4

Complementarity of epitope-specific immunotherapy. Epitope-specific therapy has been studied in Phase I/II clinical trials and in animal models. This figure depicts possible therapeutic strategies in: (1) early disease alone or with DMARDS, (2) moderate to severe disease with biologics and (3) after induction of remission.

can be lowered significantly. In rats with AA, the combination of an arthritogenic peptide (hsp60 p180–188) with one-third of the dose of etanercept led to significant improvement and immune deviation [10]. These approaches are depicted in Fig. 4.

Conclusion

It appears evident that the development gap between the evolution of molecular immunology and its application to human therapy is narrowing. The tangible outcome is a rapid evolution in the therapy of rheumatoid arthritis that is reshaping some aspects of prognosis and long-term outcomes. The main limitation remains the substantial inability of current therapies to maintain remission without continued treatment. It is probable that the development of a novel class of drugs with the ability to induce and maintain tolerance will be paralleled with the notion that combination therapy appropriately tailored to patient subpopulations may hold the promise of a less costly and more effective therapy.

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