CHAPTER 1

DEVELOPMENT OF NOVEL IMMUNOMODULATORY DRUGS AND IMMUNOTHERAPEUTIC STRATEGIES

Özgür Gündüz, MD

Recent advances in our understanding of the molecular basis of inflammatory and oncogenic processes provided the researchers with new targets for the development of new drugs. Many new cells participating in inflammation, immunity, such as innate lymphoid cells (ILCs), regulatory T lymphoids (Tregs) are defined in recent years and key roles of various related cytokines in many diseases are appreciated, shedding light on their previously obscure parts of pathogenetic mechanisms. Due to current advanced bioengineering techniques, more effective drugs find their way continiuosly into clinical use, the most popular of them being the artificially obtained monoclonal antibodies, the so-called "biologics".

BIOLOGICS IN DERMATOLOGY

What are the "Biologics"?

The term "biologics"define the molecules, monoclonal antibodies, generated by living cells through strict biotechnological interventions. They are the end result of "hybridoma technology". This technology, invented by the Nobel prize laureates César Milstein and Georges J. F. Köhler1, enable today's researchers to produce very specific antibodies against selected antigenic targets, which are designed to imitate or inhibit natural proteins in living tissues, such as cellular receptors, interleukins or antibodies.

Creation of such molecules requires fusion of two different cells; an antibody-producing B cell and the clonal cells of a specific myeloma cell line lacking the hypoxanthine–guanine phosphoribosyl-transferase gene. The initial step is to expose a laboratory animal, preferably mice, to the antigen of interest by repeated injections and force them to mount an immune response including antibody production against the injected antigen. After the immunization of mice, their splenocytes are isolated from their spleen and the myeloma cells are fused with immortalised myeloma cells by means of electrofusion[2] to obtain the so-called "hybridoma" cells. These hybrid cells are then placed in Hypoxanthine - aminopterin - thymidine (HAT) medium for an incubation period of 10-14 days. Due to blockage of nucleotide synthesis pathways by aminopterin, the unfused myeloma cells die and the fused myeloma cells, the ones who gained the ability to synthesize nucleotides through this fusion, survive. These survivor cells are immortal and are able to produce antibodies. The last step of the procedure is to identify the cells producing the appropriate antibody. The incubated cells are distributed into the wells of multi-well plates after dilution. The distribution is arranged so that every well contains only one cell, enabling the monoclonal antibodies to be collected "in seperate wells. Then, hybridoma cells in every well are incubated with an enzyme labeled antigen and a chromogenic substrate for the enzyme to visualize an expected antibody – antigen reaction. Coloration of a well indicates the hybridoma cell clone with the capability to produce antibodies against the antigen in question.

Due to this technological advances, researchers now are able to create immortal cells with the antibody-producing ability against any molecule in question, including the many cytokines, which (or roles of which) are recently defined in the pathogenesis of various dermatological diseases, such as psoriasis, atopic dermatitis, etc.

Advances in our understanding of psoriatic pathogenesis

Psoriasis is a common[3] chronic inflammatory of skin and joints, which is also suspected to have pathogenic effects on other systems.[4] Although the exact nature of etiologic factors stil eludes us, recent studies have shown immune-mediated, proinflammatory cytokine driven mechanisms in psoriatic pathogenesis[5]. Release of various inflammatory cytokines triggered by an enviromental factor has been suggested and shown by different research groups.[6,7] Interleukin (IL)-17-producing $CD4^+$ T helper (Th) cells (Th17) are current focus of attention of psoriasis researchers. Current opinion for psoriatic pathogenesis is that it starts with the recruiting of myeloid dendritic cells by activated keratinocytes.[8] Current data from various studies point out the potential role of the protein "chemerin" in psoriatic pathogenesis.[9,10] These activated dendritic cells release in turn interleukin (IL) -12 and 23, which have been shown to have prominent roles in differrentiation of T Helper cells in (Th) 1 pathway and into Th17

TABLE 4. Small-molecule inhibitors

Drug Name	Type	Target	Mode of Action	Approved since
Apremilast	Small molecule	Promoter regions of Phosphodiesterase 4 coding genes	Increases the intracellular cAMP levels by PDE4 inhibition, Inhibition of proinflammatory cytokine synthesis	March 2014 for psoriatic arthritis
Tofacitinib	Enzyme Inhibitor protein	Janus Kinase (JAK1, JAK2)	Inhibiton of JAK, modulation of JAK/STAT pathway	Phase II trials

studies, apremilast was found to be effective than placebo, but rate of PASI 75 responses in the psoriasis study at the 16th week was lower than the afforementioned biologics.

Tofacitinib is a Janus Kinase(JAK) inhibitor, which has been recently approved for the treatment of rheumatoid arthritis. JAKs are intracellular tyrosine kinases, and are part of of the Janus Kinase - Signal Transducer and Activator of Transcription (JAK- STAT) pathway. JAK-STAT pathway acts as an second messenger system and transmits signals from the receptors located on the cell membrane to the cell nucleus, to the gene promoters, altering the transcription status.[52] Tofacitinib has been shown to be effective in rheumatoid arthritis (RA)[53] and is approved for the treatment of RA. Tofacitinib is also being evaluated for its efficacy and effectiveness in psoriasis. Results from various recent phase II trials are promising[54 – 56] and reports indicate a rapid response to tofacinitib in plaque type psoriasis. There is even a case report regarding the remission of both alopecia areata and psoriasis in a patient.[57]

Summary

In recent years, new anti-psoriatic agents with very good response rates have been steadily emerging more than before. Although none of them are curative, these new molecules provides faster and longer remissions. Advances in our knowledge of psoriatic pathogenesis made it possible for pharmaceutical companies to design specific agents and to avoid the serious-side effects of the establishd drugs. Of these new drugs, IL-23 inhibitors and small-molecule inhibitiors seem to be promising candidates for therapy for psoriasis.

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