ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LXI, N1, 82 SECTIO D 2006

Chair and Department of Dermatology, Department of Clinical Immunology
Students' Research Group at the Department of Dermatology
Medical University of Lublin

EMILIA CHADAJ, AGNIESZKA KRAKOWSKA,
ANNA MICHALAK-STOMA, DANIEL PIETRZAK, ALDONA PIETRZAK,
DOROTA KRASOWSKA

Galiximab and CTLA-4Ig in psoriasis treatment

Psoriasis is a chronic and incurable skin disease which contributes to a decrease in life quality of patients and often accounts for a serious problem for physicians conducting its treatment (17). Former research does not explain the pathogenesis of psoriasis or the etiological factors of the pathological process (6, 3, 17). There were considered biochemical causes of excessive epidermis proliferation such as disorders in protein metabolism, nucleic acids, carbohydrates and lipids (13). Another hypothesis was postulated that psoriasis is a primary keratinization disorder of epidermis (3). In the last twenty years a theory that psoriasis is a disease induced by T lymphocytes has been accepted (17).

Afterwards it turned out that there are two types of T helper lymphocytes – type 1 and 2. Due to this fact the theory of the key role of T lymphocytes in the process of the disease has been verified and psoriasis got to be associated with type 1 lymphocyte (Th1 lymphocytes) with a dominating role of interferon (IFN-γ) (3). Subsequently, in the course of psoriasis there were observed vascular disorders and an inflammatory process along with elements of immunological disease (13, 6). The most recent theory displays psoriasis as a state conditioned by dysregulation of organism's innate immunity. This hypothesis concerns hyperactivity of NK cells, dendritic cells, neutrophils and keratinocytes inducing recruitment and activation of Th1 lymphocytes. It is still not proved that immunological process in epidermis has its beginning in auto-antigens (3, 6). It is known, however, that the key role in psoriasis is played by T lymphocytes. They are activated at extracutaneous areas like tonsils, lymph nodes, from where they migrate to the skin and stimulate keratinocytes to proliferate via cytokines (17). T lymphocytes' activation is a complex process, characterized by specified and sequenced occurrences, which requires an interaction with antigen presenting cells (APC). Full activation and proliferation of T lymphocytes entails at least two signals. The first of them with the recognition, uptake, internalization and conversion of the exogenous antigen by dendritic cells (14). The second signal, antigen independent, is sent by various costimulating molecules. It is transmitted by numerous interactions between the surface of T lymphocytes and the surface of the APC, which are matching receptor-ligand pairs (14). Some of the costimulating pairs in these complex relations function as adhesive molecules and are fundamental for the activation and maintenance optimal proliferation of T cells and synthesis of cytokines (14). Simultaneous delivery of both signals is decisive of primary activation of T lymphocytes. The cells that received only the first signal cannot undergo full activation and may become functionally inactive or enter the state of anergy (14).

For many years the treatment of psoriasis has been drug based which though lessened the symptoms, still did not change the mechanisms of the disease pathogenesis (17). At present, immuno-therapy is widely applied in treating different varieties of diseases, mainly including neoplasmatic diseases, either as monotherapy or in combination with chemo- and radiotherapy (13, 11). Immuno-therapy may be divided into active, passive and adoptive. The curative effect of active immunotherapy is obtained by reinforcing the patient's immune response. This is acquired

either by administering antigens to the patient (active specific immunotherapy) or activating immunological mechanisms with immunostimulating preparations like cytokines (active unspecific immunotherapy). Passive immuno-therapy is based on administering antibodies (mainly active monoclonal antibodies) which are often modified to achieve the therapeutic effect. Adoptive immunotherapy is established by introducing cells of immunological system to the patient's organism. For better healing result they are frequently activated extracorporeally (13).

The most commonly used antibodies are monoclonal antibodies, which are immunoglobulins directed against soluble or membrane related molecules that take part in the immune response (10). These antibodies of previously defined specificity are produced by cellular hybrids. The most frequently used hybrids are those gained as a result of the lymphocytes fusion (splenocytes) immunized (mice, rats) with mouse or – seldom – human myeloma cells. Selection of cells which underwent hybri-dization and verification of the specificity of antibodies produced by them, is followed by augment-tation of the selected cell clone (hybridoma). It produces chosen antibodies of programmed specificity. Fragments of antibodies are gained by enzyme digestion. They were hoped to reduce the risk of provoking the formation of human antibodies directed against allogenic monoclonal antibodies, as well as to shorten the time span of blood stream circulation before the intended combination with antigen. Owing to genetic engineering, fragments of antibodies gained by enzyme digestion are more frequently replaced by antibodies which are a mosaic of immunized human and animal protein chains. They are labelled chimerical or "humanized" antibodies (12). Humanized monoclonal antibody consists of approximately 5% of murine antibody, which consists of part $F(ab)_2$ binding the antigen and approxi-mately 95% of human IgG with parts of both light chain and heavy chain γ (Fc). These manipulations are the cause of very weak immunogenicity of humanized antibodies, which leads to the lack of patient's response to mouse protein determinants and thus allows repeated administration (6). Recently PRIMATIZED®-anti-B7 antibody has been introduced for the treatment of inflammatory and autoim-mune diseases such as rheumatoid arthritis and psoriasis. This monoclonal antibody includes variable antibody part of primate mammal (*Cynomolgus macaque*) and constant component of man (7, 16).

At present the research is being performed with the aim of assessing the efficiency of the monoclonal antibodies in the treatment of psoriasis. It is supposed that intervention in particular chains of the pathogenic mechanisms may eradicate the disease process. Most of the molecules engaged into pathomechanism of psoriasis are polypetides and protein against which the monoclonal antibodies may be produced. At present there is a number of employed antibodies where drug name suffix helps to identify the type of molecule: ximab: chimeric monoclonal antibody; zumab: humanised monoclonal antibody; umab: human monoclonal antibody; cept: protein pathway receptor-antibody, fusion protein (17, 10).

Three of the above – mentioned costimulation routes are tremendously important for inducing the specific profile of immune response in T cells: CD2/ antigen connected with the activity of 3 lymphocyte (LFA-3), LFA-1/ ICAM-1 and CD28/ B7 51. The fourth costimulation route concerning molecules CD40/ CD40 ligand 53 has recently been discovered. These routes are crucial for developing new methods of treating psoriasis like blocking the interaction between one or a higher number of the afore-mentioned receptor-ligand pairs may prevent the T cells' activation in primary response independently of the initiating antigen (14). CD80 (B7-1) and CD 86 (B7-2) belonging to the family of costimulating molecules B7 (10, 12) secure the second signal required for T lymphocytes activation. It is independent and has rather small affinity for attaching itself to CD28 receptor which undergoes constitutive expression on unactivated T cells and on most T cells in psoriatic lesions (8, 15). CD80 (B7/BB-1 – B-lymphoblast antigen-1) was exhibited for the first time in 1982 on lymphoblastic B cells activated by Epstein-Barr virus. In the following years the expression of these molecules was affirmed on stimulated monocytes, macrophages, dendritic cells, Langerhans' cells, fibroblasts, eosinophiles, T and B lymphocytes, bronchi and stomach epithelial cells and neoplastic cell lines (15).

Galiximab is a chimeric (a protein chain mosaic of immunized animals and people) anti-CD80 IgG1 lambda immunoglobulin. It is a monoclonal antibody consisting of two elements: constant human portion a and variable component derivating from primate apes (*Cynomologus macaque*).

Structurally, it is undistinguishable from human antibodies; therefore, there is very small probability that it is immunogeneic for humans. Galiximab may also be utilized in the therapy of the following autoimmune diseases: systemic lupus, Crohn's disease, idiopathic thrombocytopenia, multiple sclerosis, graft-versus-host disease, graft rejection and *alopecia areata*. It is also currently introduced in treating lymphoma (4, 16).

Biochemical grounds for signal transmitting depending on CD28 are unknown (16). It has been observed that Galiximab binds CD80 on T cells and blocks CD80-CD28 interactions without interfering with the CD80 and CD152 interaction (CTLA-4; unpublished observations). Full activation of T cells is enabled by costimulation of accessory cells. Some surface molecules are known to be able to provide the additional signal for T cell activation. The second signal for activation is created via interaction between CD28 particle, displayed on CD80 lymphocytes, and CD86 molecule displayed on APC. CTLA-4 (cytotoxic T-lymphocyte antigen-4) inhibits T cell's activation by blocking CD80/CD86 pathway. However, the signals depending on CD28 differ from the signals that depend on T cell receptors for antigen and thus are unaffected by immunosuppressive drugs like Cyclosporin and Tacrolimus (16). CD molecules are situated on the T cell's surface, including psoriatic lymphocytes. CD80 and CD86 molecules activate T cell attaching themselves to CD28 particle. The family of B7 molecules in APC regulates the activation of T lymphocytes by delivering antigen-independent, stimulating signals via CD28 and inhibiting signals via CD152 (CTLA-4) (1, 2).

CTLA-4Ig is a soluble chimeric protein. It is a fusion protein containing extracellular portion of human CLTA-4 molecule and a fragment of Fc IgG1 which constitutes a new class of biological factors (5). CTLA-4Ig (BMS-188667) includes in its structure extracellular domain of human CD152 and fragment (CH3 and CH2 domain attachments) of Fc part of human IgG (1). CTLA-4Ig is a biological particle which blocks the second signal required for T cells' full activation in the immune response (5).

The first report on treating 24 patients suffering from plaque psoriasis (moderate and severe chronic plaque psoriasis) with anti-CD80 monoclonal antibody (Galiximab) was published in 2002 (7). The percentage of skin lesions in the examined persons amounted to 10%, severity scale of the course of psoriasis amounted to 6 points, Psoriasis Area and Severity Index (PASI) on average amounted to 22.9. IDEC-114 (anti-IgG1 monoclonal antibody) was examined. Special attention was paid to safety, pharmacokinetics and clinical activity. A single rising dose was administered (0.05 mg/kg, 0.25 mg/kg, 1 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg). The authors assessed that a single dose of IDEC-114 is safe, well tolerated and improves the clinical state of the skin. No serious side-effects were observed. Systemic ailments were developed such as: mild asthenia (29% of subjects), chills (25%), headache (21%), dizziness (17%), fever (13%) and infections (13%). 29% of complaints occurred on the day of drug administration. The best improvement according to PASI was noted in the group taking Galiximab (IDEC-114) in the dose of 10.0 mg/kg. The serum half-life of IDEC-114 was approximately 13 days.

Another examination performed by Gottlieb et al. encompassed 35 patients (in 5 centers of the USA). The average index PASI amounted to 19.6. Seven groups were created, including 5 persons each (8). Galiximab was administered intravenously according to three various schemes, in different doses and four infusions of 2.5 mg/kg, 5.0 mg/kg or 10.0 mg/kg during one day weekly. Two groups were administered four infusions of 5.0 mg/kg or 10.0 mg/kg every two weeks. Two next groups were administered Galiximab in the following doses: the first group – first infusion of 10 mg/kg and the next three of 5.0mg/kg; the other group – the first infusion 15.0 mg/kg and the next three 7.5 mg/kg. The majority of the patients displayed reduction in PASI index in comparison with the initial value. Fourteen (40%) of patients displayed regression of psoriatic lesions and reduction in PASI index to approximately 50%, while with the following 4 patients (11%) the reduction reached 75%. On day 71 of the examination a 50% reduction in PASI index compared to the initial state was achieved with 8 out of 35 patients (23%) and on day 127 with 10 out of 35 subjects (29%). Improvement of the clinical state judged according to Physician's Global Psoriasis Assessment (PGA) was noticed in over 57% of cases (PGA good grade) and 20% of subject achieved perfect results. The development of therapy was assessed according to Psoriasis

Severity Scale (PSS). The initial average of PSS amounting to 7.6 lowered on day 127 of examination to 5.0.

During Galiximab treatment 8 subjects received other antipsoriatic drugs simultaneously. Three subjects underwent general treatment or phototherapy, while 5 subjects underwent local treatment. The last 5 displayed varied response to the treatment: PASI index exhibited deterioration in 2 subjects and 3 other showed improvement; PGA deteriorated with one subject, 3 subjects exhibited improvement and one subject did not change.

Similarly to the first examination that was carried out with single doses slight side-effects were observed in 74% of the diseased: infections in 29%, pruritus in 17%, chills in 11%. Nine percent of subjects exposed symptoms like: asthenia, fever, headache, pain, dizzness, rhinitis. Galiximab half-life in plasma was from 14 to 19 days. The most important information provided by the authors is a complete lack of immune response to Galiximab. Cytokine-release syndrome or a decrease in T cells and B lymphocytes number described in the literature were not displayed. In histopathological examination of biopsies taken from psoriatic changes improvement in all groups of subjects was exhibited. A dose and response relationship was not found though (8).

Results of the research carried out by Gottlieb et al. (7, 8) acknowledged the hypothesis that Galiximab may act by blocking CD80-CD28 pathway, allowing for deactivation of T cells by CD80-CD152 interaction and/or complement dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC).

Clinical trials of treating psoriasis with BMS-188667 preparation, i.e. CTLA4Ig, conducted by Abrams et al. (2) were preceeded by *in vitro* experiments (1, 2). It has been found that CTLA4Ig inhibits dose-dependently the ability of B7 molecules, presented on Langerhans' cells and dermal dendritic cells, to act as molecules costimulating the proliferation of T cells in primary immune response. Abrams et al. (1) assessed the efficiency of intravenous infusions in repeated doses in 4 consecutive pulses of CTLA4Ig preparation in 43 persons suffering from psoriasis. The pulses were administered in 5 consecutive weeks: on day 1 (week 1), day 3 (week 1), day 16 (week 3), day 29 (week 5). The average body surface taken by the pathological process amounted to 25% in the range from 11 to 55%. The patients were divided into 8 groups, depending on the administered dose. Each group consisted of 4–6 persons. The first dose amounting to 0.5 mg/kg was identical for all groups. Then the following groups received, accordingly: 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 4 mg/kg, 8 mg/kg, 16 mg/kg, 25 mg/kg and 50 mg/kg. Confronted with the initial state of psoriasis 19 out of 41 patients (46%) acquired 50% clinical amelioration of the disease in PGA assessment. Very good clinical effects reflected in a 50% improvement in all clinical parameters of psoriasis were observed in 9 out of 11 subjects receiving the largest doses of CTLA4Ig and in one out of 9 subjects belonging to groups receiving the smallest doses. According to the assessment performed on day 147 (week 26), 9 subjects out of 11 achieved $\geq$ 50% sustained improvement of the clinical state of psoriasis (1). CTLA4Ig was well tolerated by the majority of patients and occasionally the number of circulating lymphocytes was lowered (1, 17). Despite this fact cases of immunosuppression parallel to oportunistic infections and neoplasms were not found clinically. The most frequent side-effects found during the period of observation (average 176 days) were mild. They consisted of mild infection of the respiratory tract (16%) and passing headaches (16%). Histopathological examination displayed returned proper skin image.

Summing up, it is worth noting that many new biological drugs cause significant clinical amelioration in the research groups. Due to new biological immune response modulators in monotherapy or in combination with fixed treatment with e.g. methotrexate more individualized and conditionally safer treatment will be possible (17). "Biological drugs may be compared to marksmen whose role on a battlefield is to precisely eliminate targets but the consequence of their actions may be compared to massive artillery action (which resembles traditional immunosuppressive anti-psoriatic drugs)" (10).

In recent years the treatment of autoimmunological diseases is based on biological drugs. Remote complications and the dangers of administering the humanized monoclonal mouse antibodies, which very specifically influence the immune and antineoplasmatic response (6), are unkown. Due to our economic situation therapies based on monoclonal antibodies, fusion proteins,

soluble receptors, cytokines and anti-cytokines seem out of reach for routine treatment of psoriasis (6). Drugs neutralizing biological TNF activity such as Infliximab, Etanercept, Adalimumab, used in rheumatoid arthritis did not lead to permanent recovery. In the case the treatment was discontinued for longer than 3 months it inevitably meant the relapse of disease (9). There is no evidence in the literature on recurrences of psoriasis treated with the help of the above-mentioned preparations.

REFERENCES

1. Abrams J.R. et al.: CTLA4Ig-mediated blockade of T-cell costimulation in patients with psoriasis vulgaris. J. Clin. Invest., 103, 1243, 1999.
2. Abrams J.R. et al.: Blockade of T lymphocyte costimulation with cytotoxic T lymphocyte- associated antigen 4-immunoglobin (CTLA4Ig) reverses the cellular pathology of psoriatic plaques, including the activation of keratinocytes, dendritic cells, and endothelial cells. J. Exp. Med., 192, 681, 2000.
3. Bos J.D. et al.: Psoriasis: Dysregulation of innate immunity. Br. J. Dermatol., 152, 1098, 2005.
4. Czuczman M. S. et al.: Phase I/II study of Galiximab, an anti-CD80 antibody, for relapsed or refractory follicular lymphoma. J. Clin. Oncol., 23, 4390, 2005.
5. Filipowicz-Sosnowska A.: Współczesne i przyszłościowe terapie biologiczne w reuma-toidalnym zapaleniu stawów. Med. Dypl., 13, 26, 2004.
6. Gliński W.: Czy terapia białkami fuzyjnymi i humanizowanymi przeciwciałami monoklonalnymi ma przyszłość w łuszczycy? Dermatologia, 4, 27, 2002.
7. Gottlieb A.B. et al.: Clinical and histologic response to single-dose treatment of moderate to severe psoriasis with an anti-CD80 monoclonal antibody. J. Am. Acad. Dermatol., 47, 692, 2002.
8. Gottlieb A.B. et al.: Evaluation of safety and clinical activity of multiple doses of the anti-CD80 monoclonal antibody, Galiximab, in patients with moderate to severe plaque psoriasis. Clin. Immunol., 111, 28, 2004.
9. Kalińska I.: TNF-alpha blockers therapy in rheumatoid arthritis. Probl. Lek., 42, 288, 2003.
10. Kwiek B., Langner A.: Leki biologiczne w leczeniu łuszczycy – futurystyczna wizja czy praktyka dermatologa. Dermatologia, 2, 74, 2005.
11. Nurzyński P.: Immunoterapia nowotworów jelita grubego. Nowa Klin., 4, 410, 1997.
12. Obłąkowski P.: Radioizotopy sprzężone z przeciwciałami monoklonalnymi w radioimmuno-scyntygrafii i radioimmunoterapii. Klinika. Immunologia, 5, 48, 1995.
13. Pietrzak A.: The immunological possibilities of treatment of psoriasis. Alergologia, 1, 50, 2002.
14. Prinz J.C.: The role of T cells in psoriasis. J. Eur. Acad. Dermatol. Venerol., 17, 257, 2003.
15. Rutkowski R., Moniuszko T.: The role of costimulatory molecules B7.1 (CD80) and B7.2 (CD86) in the pathomechanism of inflammatory process. Alerg. Astma Immun., 6, 87, 2001.
16. Schopf R.E.: IDEC-114 IDEC. Curr. Opin. Investig. Drugs, 2, 635, 2001.
17. Young H.S., Griffiths C.E.M.: Advances in immunotherapy for psoriasis. G. Ital. Dermatol. Venerol., 138, 349, 2003.

SUMMARY

In the past decade a new branch of therapy for psoriasis called biologic therapy has developed. Mechanisms of blocking various steps of immune reactions and blocking CD80/CD86 and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) are used in the therapy. Authors

present a literature review concerned with psoriasis treatment with Galiximab and CTLA4Ig, as well as adverse effects of these agents.

Galiximab i CTLA-4Ig w leczeniu łuszczycy

W ostatnich latach w leczeniu łuszczycy obserwujemy rozwój nowej gałęzi terapii leków tak zwanych „biologicznych". Wykorzystywane są mechanizmy blokowania różnych etapów reakcji im-munologicznych, blokowanie cząsteczek CD80/CD86 i antygen 4 związany z limfocytem T cytotoksycznym (CTLA-4). Autorzy prezentują przegląd piśmiennictwa dotyczący leczenia łuszczycy za pomocą galiximabu i CTLA4Ig oraz działań ubocznych związanych ze stosowaniem tych preparatów.