LETTER TO THE EDITOR

CARCINOMA CUNICULATUM IN COURSE OF ETANERCEPT: BLOCKING AUTOIMMUNITY BUT PROPAGATION OF CARCINOGENESIS?

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Carcinoma cuniculatum (CC) or verrucous squamous cell carcinoma is a rare variant of squamous cell carcinoma with low incidence of metastasis. It mainly affects men during the fifth-sixth decade of life, arising mostly on the weight-bearing surface of the foot, but it can also be found in other body areas. The favorable effects on the psoriatic, rheumatoid, juvenile polyarthritis as well as the ankylosing spondylitis after the application of Tumour Necrosis Factor (TNF)-alpha inhibitors, like etanercept, presume the availability of similarity between the etiopathogenetic mechanisms which are responsible for the generation of the inflammatory cascade. According to the latest studies, the sensitivity of the patients to TNF-alpha inhibitors could be genetically determined and may also be due to certain genetic polymorphisms of the NLP3 and CARD8 zones of the inflammasome. The blocking of the inflammatory reaction within the borderlines of the psoriatic arthritis could also be accepted as something of a "double-edged sword". There is a growing volume of literary data which informs us of the clinical manifestation, not only of skin, but also of other types of tumors after the application of TNF-alpha inhibitors. This inevitably generates the hypothesis that within a certain group of patients the TNF-alpha inhibitors have some additional, and currently obscure, effects on presumably key regulatory proteins of the so-called extrinsic apoptotic pathway. Other proteins of the human inflammasome could be also implicated in the regulation of the programmed cell death and the carcinogenesis - there are speculations, that the adapter protein, ASC/TMS1, could be one of these. The present study describes the case of a patient who developed a rare form of skin tumor - epithelioma cuniculatum - whilst undergoing etanercept therapy for psoriatic arthritis. Under discussion are the possible critical connections in the complex regulatory "networks" of the inflammatory processes, the programmed cell death (apoptosis) and the carcinogenesis which, in the near or distant future, could become the objects of a targeted therapy.

The pro-inflammatory cytokine Tumour Necrosis Factor (TNF)-alpha has a key role in the pathogenesis of various infectious and inflammatory disorders (1). Clarification of its pivotal role in the pathogenesis of rheumatoid arthritis, psoriasis, psoriatic arthritis and inflammatory bowel disease has resulted in the successful development of TNF-blocking options (1). For this reason, data on the concurrence of

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several chronic inflammatory diseases have led to the hypothesis of common pathogenetic processes of cytokine dysregulation (1). It is believed, that this cytokine superactivation, or deregulation, has an impact on the different areas of the so-called inflammasome, but also of the extrinsic apoptotic pathway (2, 3).

A number of literary sources offer information on the participation of inflammasome components in the activation of JIA but also in the case of rheumatoid arthritis (2, 4). The changes effecting the inflammasome may, in most general terms, be divided into genetic and epigenetic (5).

In the case of the epigenetic changes, the investigations of the effects (direct or indirect) of the TNF-L inhibitors on certain components of the inflammasome are forthcoming. Their additional influence on key adapter proteins, like ASC/TMS1, the proapoptotic proteins BAX, BAK, BID and the regulator of the cell cycle, p 53, is of particular importance (5).

One of the latest studies published found solid evidence of modulation of the NLRP3-inflammasome in patients with RA prior to receiving infliximab and some evidence of association for SNPs at NLRP3 and CARD8 loci with RA susceptibility and response to anti-TNF (2).

Other data indicate that the rs4353135 OR2B11/ NLRP3 polymorphism might be functional in, and could contribute to, the pathophysiology of oligoarticular and polyarticular JIA in a Taiwanese population (4).

The similarity in the therapeutic results when applying etanercept in the treatment of the rheumatoid arthritis, axial spondyloarthritis, psoriatic arthritis and juvenile idiopathic arthritis should suggest, at least to an extent, similar etiopathogenetic mechanisms in the above-described diseases and the generation of the inflammation (6). On the one hand, these mechanisms should be connected with the inflammatory regulators, but on the other – and most probably – with the key proteins which regulate the processes of the programmed cell (apoptosis) and the blocking of the cell cycle (3). These are mechanisms which form the base for the development of skin and other neoplasms, described in a number of literary sources (3, 7, 8). It is assumed that one of the key regulators of the processes of cell cycle regulation, apoptosis and also the regulation of the human inflammasome could be the key adaptor protein ASC/TMS1 (3 ,5).

Case report

A 57-year-old woman was referred to our unit with a 12-month history of a painful, non-healing lesion on the posterior aspect of her left heel. She had attempted to remove a long-standing callus at the site with several cycles of cryotherapy, diathermocoagulation and surgical curettage without success. The lesion recurred and macerated due to secretion of large amounts of malodorus material. Secondary bacterial infection of a wart was presumed by the general practitioner so three courses of antibiotics (minocycline, amoxicillin clavulanate and azithromycin) were administered with potassium permanganate 1/8000 solution topically, but with no significant clinical improvement.

The patient, who also suffered from psoriatic arthritis (with previous disabling low-back pain, arthritis of the right knee and dactylitis of the I, II and III digits of both hands) presented with nail pitting and minimal psoriatic skin lesions (PASI score: 2) on scalp, elbows and sacral region. Her medical history reported 'callosities' of both feet, but no family history of keratoderma or skin cancer.

She was treated by methotrexate for three years, and etanercept 25 mg subcutaneously weekly over the previous year, with satisfactory control of the rheumatic disease.

Examination of the left foot revealed a well circumscribed, cauliflower-like hyperkeratotic and ulcerative mass, measuring approximately 3 x 4 cm, with a 'spongy' appearance and a foul odour (Fig. 1). No bony prominences or gross deformities were noted. The patient had pain and some limitations in walking. On palpation, no inguinal lymphadenopathy was evident. Pedal pulses were present.

A wedge biopsy was performed, histological examination being inconclusive but showing atypical squamous proliferation (Fig. 2). Based on suspicion, magnetic resonance imaging was ordered and no bony involvement was noted. Wide excision of the lesion was then performed and sent to pathology for direct evaluation. After confirmation of clear margins, the wound site received full thickness skin graft taken from the anterior hip crease.

Histology revealed the presence of hyperkeratotic spindle-cell proliferation with deep endodermal papillar digitations, without infiltration or significant nuclear atypia (Fig. 2). Based on histopathological findings, the diagnosis of carcinoma cunniculatum was made. A diagnostic work-up including inguinal lymph nodes and liver ultrasonography was unremarkable.

The patient did not respect any of the follow-up steps.

Through high clinical suspicion, accurate anamnesis and subsequent histopathological examination, bone involvement and amputation may be avoided.

DISCUSSION

CC is a rare variant of verrucous carcinoma that was firstly reported by Ackerman in 1948 as a distinct low-grade malignancy (9). In 1954 Aird et al. introduced the term "carcinoma cuniculatum" to describe a cutaneous verrucous carcinoma that extends into underlying tissues with a peculiar histologic pattern in which branching and interconnecting tunnels and clefts invade deeply in several directions, resembling a 'rabbit warren' (10).

With regard to our case, the lesion was atypically located on the posterior aspect of the heel; the particular site solicited the suspect of a proliferative process instead of the 'classical' verruca vulgaris presentation, so further medications or physical procedures were avoided. Moreover, because of previous treatments, the patient was not so prone to further attempts without a clear diagnosis.

There is an increased risk of melanoma and also non-melanoma skin cancer in patients treated with TNF-alpha inhibitors (3,11). The risk for non-melanoma skin cancer is increased 2 to 3 fold by TNF-alpha inhibitors (11). Anti-TNF-alpha was stopped according to guidelines (12).

The apoptotic machine in the keratinocytes is probably responsible for the activation of the extrinsic apoptotic pathway, which seems to be a connection to the processes of immunosurveillance, because at that way the immune system could be additionaly activated with the purposed to eliminate the already existing tumour or dysplastic cells (3, 13). The mitochondrial or intrinsic and the death ligand mediated, extrinsic apoptotic pathway seems to be the most important pathways for the integrity of genome and the elimination of the neoplastic cells (3,13). Death receptors 4 and 5, known also as DR4 and DR5 are cell surface receptors that could be activated by their tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), that could trigger apoptosis in most cancer cells in BCC and SCC, but not in normal cells (14). It has been suggested that the extrinsic apoptotic pathway is also linked to immunosurveillance (14). The blocking of this process with certain medical drugs could lead to the clinical manifestation of various cancer forms, including NMSC (3). The "potentiating" of this branch of the apoptosis should be able to destroy the tumor cells. Certain polymorphisms of the so called death receptors, like TRAIL or death receptors (DRs), could define the varying sensitivity of the different patients to anti-TNF alpha receptor antagonists such as etanercept, adalimumab, infliximab, which find an application in patients with various autoimmune processes (15).

According to the most-modern understanding, from the point of view of etiopathogenetics the regulation of the inflammation and the carcinogenesis appear to be inextricably bound, very complicated and, at the same time, complex processes (3, 5). Chronic inflammation is, on the one hand, capable of agitating the carcinogenesis and on the other, in some cases the inflammation itself is desirable for the elimination of the residual and potentially dangerous pre-tumor or already dysplastic cells (3,5).

The ASC/TMS1 protein activates the inflammasome and the secretion of a number of key cytokines like IL/1 ß and IL-18 which are responsible for the subsequent inflammatory reaction activating the phagocytes (16-19). On their part, these clean the residual material of apoptotic corpuscles and debris (5). Increased sensitivity to apoptosis and effective clearance of apoptotic bodies could prevent carcinogenesis (5). The ASC/TMS1 adapter protein has an indirect pro-apoptotic effect via its interaction with p53 and BAX (3,5).

Bax protein could bind to the PYD domain of ASC/TMS1 (3,5). ASC/TMS1 could act also as a carrier protein of BAX by translocating it to mitochondria (5). Another study came to the conclusion that the Bax dependent apoptosis could

be linked to the activation of caspase 8. ASC/TMS1-mediated apoptosis was also activated by the caspase 8-induced maturation of BID protein (20). BID could trigger the death receptor-activated, mitochondria-mediated apoptotic pathway (21). The inactivation of ASC/TMS1 reduced the phosphorylation of p53 and subsequently decreased the expression of its target genes, such as Bax (3, 5). Finally, all these studies imply that the reduced expression of the adaptor protein ASC/TMS1 in cancer cells can block apoptosis and potentiate carcinogenesis (3, 5). However, at the same time, the chronic inflammation can potentiate the carcinogenesis via processes which are not directly linked to the activation of the inflammasomes (3, 5).

There is a lurking hypothesis that a number of drugs from the group of TNF alpha inhibitors may exert a direct influence on the extrinsic apoptotic pathway and its activation through the neutralization of the death receptors within the framework of the extrinsic apoptotic pathway (3, 22) which seems to be a key point in the apoptotic cascades that can potentiate or eliminate cancer cells and also target the development of different anti-cancer drugs in different cancer types (23).

Association of NLRP3 and CARD8 genetic polymorphisms with juvenile idiopathic arthritis in a Taiwanese population has been also recently described (4). Evidence of NLRP3-inflammasome activation in rheumatoid arthritis (RA) has been also found, according to other literature data (2). The NLRP3-inflammasome, implicated in the pathogenesis of several inflammatory disorders, has been analysed in rheumatoid arthritis (RA) (2). The application of etanercept in the treatment of psoriatic, ankylosing and rheumatoid arthritis presumes similar etiopathogenetic concepts for both diseases. In the near future a connection will probably be found between TNF L and ASC/TMS1 (3, 5). Currently, there is convincing evidence that chronic inflammation is linked to carcinogenesis (3, 5). ASC/TMS1 is an important adaptor protein interacting with caspases via the CARD domain and inflammasome receptors via the PYD domain (5). In several type of cancers the ASC/TMS1 gene seems to be hypermethylated and desactivated (24-26). There are no data in the literature describing the methylation state of the ASC/TMS1 gene in patients

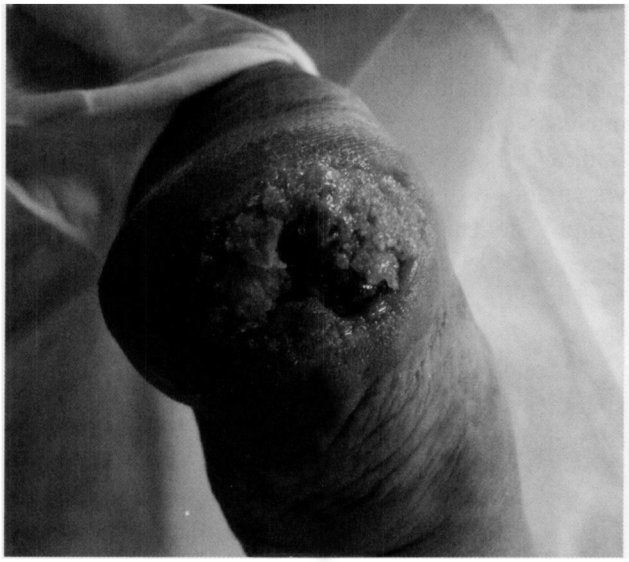

Fig. 1. *Posterior aspect of the left heel, showing, in the contest of a well defined bilateral focal keratoderma, an indurated verrucous, cauliflower-like, centrally fissured plaque with multiple openings.*

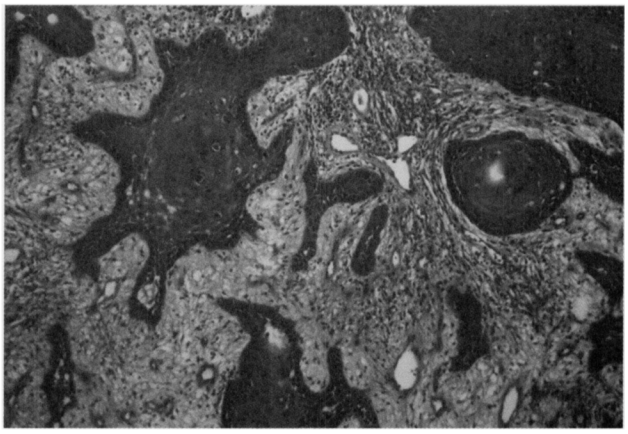

Fig. 2. *Hyperkeratotic and acanthotic, well differentiated spindle-cell proliferation with deep endophytic growth. No significant nuclear atypia was noted. (Hematoxylin and Eosin stain, magnification x50)*

before and after the treatment with anti-TNF alpha drugs like etanercept in patients with psoriatic arthritis with the purpose of elucidating the risk of the development of cutaneous malignancies. In the near future the model for such an investigation should be carefully designed and executed.

Of interest is the fact that the exposure of cancer cells to DNA methyltransferase inhibitors, such as

zebularine, 5 -aza-2'-deoxycitidine, could restore the expression of ASC/TMS1 and induce apoptosis (3, 27). The methylation status of CpG island of ASC/TMS1 gene seems in that way to be generally a sensitive prognostic marker for tumorigenesis (5). This is an indication that ASC/TMS1 is subjected to an epigenetic regulation which may lead to the discovery of new medicaments capable of exerting an impact on certain functions of the adaptor protein through the "restoration" of its functions or through demethylation.

Treatment

Surgery is the treatment of choice (12, 13, 28). Depending on local extension of the tumour, surgery may be mainly conservative, through using Mohs technique along with skin graft and flaps for larger defects (11, 14). In cases of extensive diseases 'functional' amputation of the involved segment is unavoidable, similarly to other neoplasms (29).

CONCLUSIONS

1) The fact that etanercept is capable of inducing carcinogenesis, and in particular the clinical manifestation of carcinoma cuniculatum improving, at the same time, the symptoms in patients with psoriatic arthritis is an unambiguous indication to a connection between the processes which participate in the regulation of inflammation, autoimmunity and programmed cell death –apoptosis. Doubtless, the TNF L inhibitors, like etanercept, have a more complex impact on these processes than was previously believed.

2) TNF-alpha inhibitors may exert a direct influence on the extrinsic apoptotic pathway and its activation through the neutralization of the death receptors within the framework of the extrinsic apoptotic pathway.

3) One of the key complex regulatory proteins which take an active, but also simultaneous, role in the above-described processes is ASC/TMS1. In its hypermethylated form it is deactivated while in its demethylated form it is active. The active demethylated form of ASC/TMS1 is capable of integrating with the regulator of p53 and the proapoptotic protein BAX and caspase 8. As far as the side effects from a number of immunomodulating substances are concerned, these remain to be the subject matter of future investigations.

4) The processes in the inflammasome are directly related to the regulation of the cell cycle, the programmed cell apoptosis and the inclination to the development of autoimmunity.

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