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EVOKED PAIN BEHAVIOR AND SPINAL GLIA ACTIVATION IS DEPENDENT ON TUMOR NECROSIS FACTOR RECEPTOR 1 AND 2 IN A MOUSE MODEL OF BONE CANCER PAIN

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Abstract—Bone–cancer-related pain is one of the most disabling factors in patients suffering from primary bone cancer or bone metastases. Recent studies point toward an important role of proinflammatory cytokines, example tumor necrosis factor-α (TNF), for tumor growth and bone–cancer-associated pain. Mechanisms by which TNF, through its receptor subtypes, TNF receptor 1 (TNFR1) and −2 (TNFR2), elicits altered sensation and pain behavior, are still incompletely understood. To look for a potential role of TNF in bone cancer pain, cancer-related pain was analyzed in fibrosarcoma-bearing C57Bl/6J wild type mice after systemic antagonism of TNF. To further clarify the role of TNF receptor (TNFR) in bone-cancer pain, naive and fibrosarcoma-bearing C57Bl/ 6J wild type and transgenic mice with a deficiency of TNFR1 (TNFR1ko), TNFR2 (TNFR2ko), and TNFR1+2 (TNFR1+2ko) were compared regarding cancer-related pain and hyperalgesia, tumor growth, osteoclast activation, and spinal astrogliosis. Systemic antagonism of TNF significantly alleviated tactile hypersensitivity and spontaneous bone–cancer-related pain behavior. Most interestingly, combined deletion of the TNFR1 and TNFR2, but not of either gene alone, almost completely inhibited the development of tactile hypersensitivity, whereas spontaneous pain behavior was transiently increased. Accordingly, spinal astrogliosis was markedly reduced, whereas tumor growth was significantly increased in TNFR1+2ko mice. In contrast, deletion of the TNFR1 or TNFR2 gene alone did not change tumor growth or spinal astrogliosis. Our findings suggest that the combined absence of TNFR1 and TNFR2 is necessary for the attenuation of cancer-related tactile hypersensitivity and concomitant spinal astrogliosis, whereas tumor growth seems to be inhibited by combined TNFR activation. These findings support the hypothesis of cytokine-dependent pain development in cancer pain. Differential targeting of TNFR activation could be an interesting strategy in bone–cancer-related pain conditions.

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Abbreviations: GFAP, glial fibrillary acid protein; H&E, hematoxylin and eosin; IL, interleukin; MAC, macrophage; MEM, minimum essential medium; RANKL, receptor activator of NF-kB ligand; TNF, tumor necrosis factor-α; TNFR1, TNF receptor-1; TNFR1ko, TNFR1 knockout; TNFR2, TNF receptor-2; TNFR2ko, TNFR2 knockout; TNFR1+2ko, TNFR1 and 2 knockout; TRAIL, TNF-related apoptosis-inducing ligand; TRAP, tartrate resistant acid phosphatase; TRPV1, transient receptor potential vanilloid 1; WT, wild-type.

Key words: tumor necrosis factor-α, TNF receptor 1 and 2, bone cancer pain, hyperalgesia, spinal astrogliosis, etanercept.

Cancer pain is a significant clinical problem as it is the first symptom of disease in 20–50% of all cancer patients, and 75–90% of advanced or terminal cancer patients must cope with chronic pain syndromes related to failed treatment and/or tumor progression (Mercadante and Arcuri, 1998; Portenoy and Lesage, 1999). Despite the high incidence and morbidity, the exact mechanisms of cancer-related pain are still unclear. In the past years, animal models for bone cancer pain that represent most of the clinically important symptoms of bone-cancer pain (bone destruction, ongoing and breakthrough pain) have been established (Schwei et al., 1999; Cain et al., 2001; Schweizerhof et al., 2009).

Tumor necrosis factor-α (TNF) is a proinflammatory cytokine produced by several cell types including mast cells, macrophages, fibroblasts, endothelial cells and Schwann cells (Friedman, 2000), and by certain tumor cells (Selinsky and Howell, 2000; Fukuda et al., 2001). TNF and interleukin- (IL)-1 and IL-6 protein levels are upregulated in tumor site homogenates and in the spinal cord in mouse models of fibrosarcoma implantation in the calcaneus (Wacnik et al., 2005) or tibial bone (Baamonde et al., 2007), and in a soft tissue tumor model with s.c. injection in the hind paw (Constantin et al., 2008). It is well established that cytokines play a role in tumor development and metastasis and their effects vary depending on the type and location of the tumor. TNF has been shown to induce tumor growth, promote angiogenesis, and increase metastasis by down-regulating tumor-suppressor genes and/or increasing adhesion (Dunlop and Campbell, 2000). However, TNF is also known to induce apoptosis of tumor cells, therefore limb or organ infusions of TNF have been used as an adjuvant for chemotherapy in cases of non-resectable high-grade sarcomas, melanomas, and liver tumors (for review see Ashkenazi, 2002).

Recently, it has been suggested that cytokine activation may also play a role on the induction of cancer-related pain: intraplantar injection of TNF in naive or tumor-bearing mice induced mechanical hypersensitivity, suggesting that TNF can excite or sensitize primary afferent fibers to mechanical stimulation in both naive and tumor-bearing mice (Wacnik et al., 2005). It was shown that TNF-induced cancer-related heat hyperalgesia through nociceptor sensitization is linked to upregulation of transient recep-

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tor potential vanilloid 1 (TRPV1) (Constantin et al., 2008). Systemic pre-implantation as well as local post-implantation injection of the TNF antagonist etanercept partially blocked mechanical hyperalgesia, indicating that local production of TNF may contribute to tumor-induced nociception (Wacnik et al., 2005; Constantin et al., 2008). Accordingly, systemic, but not intrathecal treatment with the IL-1 receptor antagonist anakinra blocked mechanical hyperalgesia induced by tibial osteosarcoma, suggesting that peripheral release of IL-1β is responsible for the development of pain behavior in this model (Baamonde et al., 2007).

The mechanisms by which cytokines elicit pain behavior in bone cancer pain are still unclear.

Actions of TNF are known to be mediated through two distinct receptors, the constitutively expressed TNF receptor (TNFR) 1 and the inducible TNFR2 (MacEwan, 2002). Recently, it was shown that in the soft tissue cancer model, TNFR2 deficient (ko) mice display delayed onset of thermal hyperalgesia (Constantin et al., 2008). In bone-cancer pain, the role of TNFR activation is unknown to date.

On the basis of these findings, we speculated that the release of TNF and TNFR activation may play a role on the development of pain in bone-cancer pain, possibly by effects on osteoclast activation or spinal astrogliosis. Therefore, we examined bone–cancer-induced pain behavior in wild-type mice after systemic antagonism of TNF by etanercept. Furthermore, we tested the development pain behavior in fibrosarcoma tumor-bearing TNFR1ko, TNFR2ko, and TNFR1+2ko mice compared with wild type mice. In addition, we analyzed the extent of tumor growth, osteoclast activation in the bone, and astrocyte activation in the spinal cord.

EXPERIMENTAL PROCEDURES

Animals

Experiments were performed on adult (aged 8–10 weeks, 20–30 g body weight), male mice of C57Bl/6J background. The C57Bl/6J mouse strain was syngenic to the fibrosarcoma cells used in these experiments. Thus, these cells form tumors after implantation without rejection. Mice were housed in boxes of six in a temperature- and humidity-controlled environment, with a light/dark cycle of 14:10 h with standard rodent chow and water *ad libitum*. All

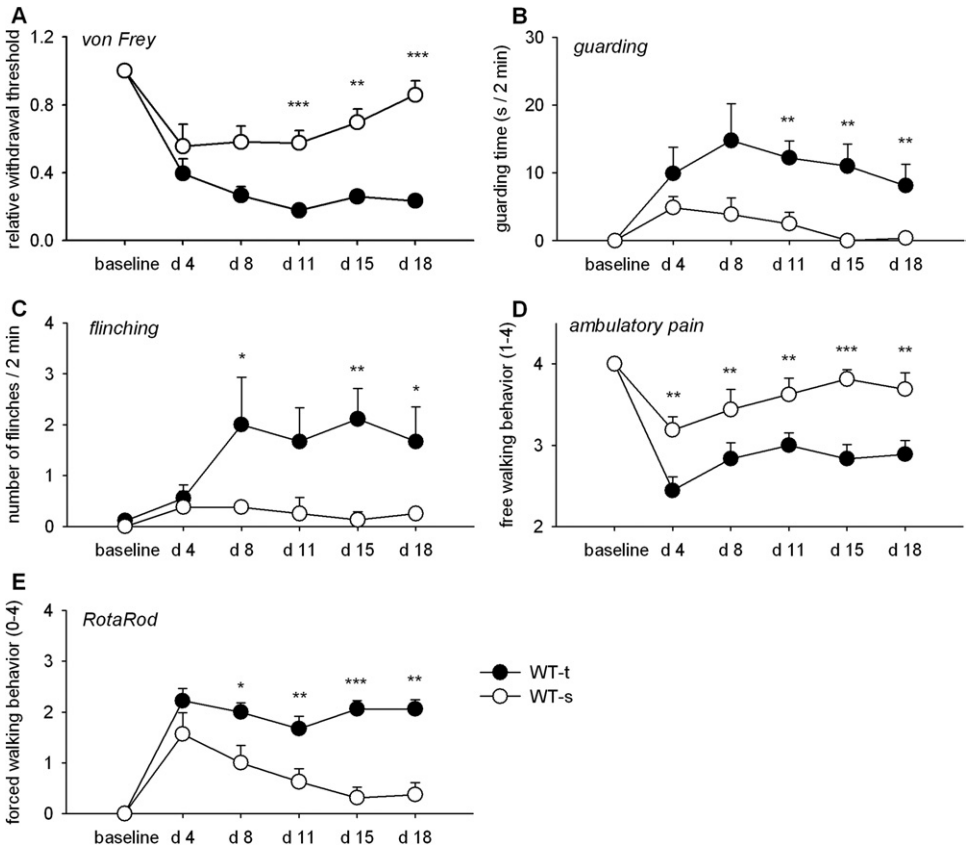

Fig. 1. Pain-behavior of WT mice with intrafemoral injections of culture medium containing 10^6 fibrosarcoma cells (t = tumor) or culture medium (s = sham). (A) Tactile hypersensitivity measured as withdrawal thresholds to von Frey filaments on the plantar surface of the ipsilateral hind paw: WT-t mice showed significant reduced withdrawal thresholds compared with WT-s mice (d11-d18). (B, C) Ongoing pain behavior was measured as the guarding time (B) and number of flinches (C) during a 2-min observation period: WT-t mice displayed increased guarding time (B, d11-d18) and increased number of flinches compared with WT-s mice (C, d8, d15-d18). (D, E) Movement-related pain behavior measured as limb use during normal ambulation (D) and guarding during forced ambulation on a rotarod. (E) WT-t mice showed impaired limb use during spontaneous ambulation (D, d4-d18) and increased guarding upon forced ambulation (E, d8-d18) compared with WT-s mice (* $P<0.05$, ** $P<0.005$, *** $P<0.001$).