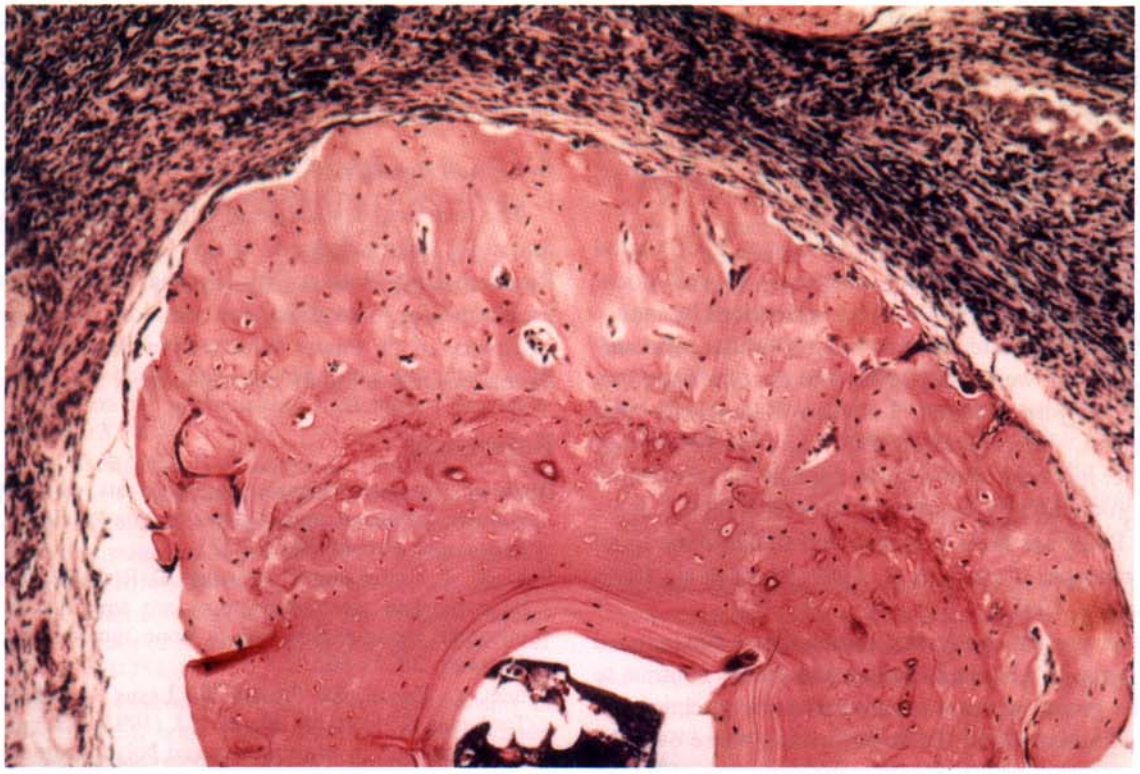

Fig. 4. Exostosis of tarsal bone, 27 days post CFA administration into the foot pad. An inflammatory reaction around the tarsal bone is still evident. Magnification 400x.

(Fig. 2). With progression of time the ossicles matured, the osteocytic lacunae, originally large, became smaller and the newly formed bone has formed a solid block together with the resident bone (Fig. 4). This newly formed bone was covered with periosteum, but no osteoclasts were apparent within it. In contrast, osteoclasts were observed frequently inside the enlarged periosteum at early stages of the pathology (7–16 days). Spaces between newly formed ossicles were colonized by bone marrow and at more advanced stages, these regions merged with the main marrow cavity. Similar changes were observed in the distal part of shank bones involved in the CFA-induced inflammatory reaction. In the lymph nodes draining the IFA-induced inflammation, examined 13 days post adjuvant administration, besides an increased number of germinal centers, mast cells were virtually absent. However, in the lymph nodes of CFA-induced inflammation, as well as in control lymph nodes, mast cells were always present and occupied all sinuses: subcapsular, radial and medullary.

Discussion

Administration of IFA and CFA induces a localized granulomatous reaction: actively growing fibroblasts and capillary buds, aggregations of macrophages surrounded by mononuclear cells, mainly lymphocytes, and foreign body type giant, multinucleated cells. So-called epithelioid cells, which are modified macrophages, are also constituents of granuloma tissue (Robins, 1974).

It was shown previously that the administration of various immunomodulators produced both, formation and resorption of local bones with variable frequency. Vaccinium antituberculosum – BCG – dissolved in the CFA stimulated mostly periosteal osteogenesis, while the administration of BCG antigens dissolved in saline stimulated mostly bone resorption (Włodarski and Galus, 1992). As the CFA contains antigens of *Mycobacterium tuberculosis*, the possibility rises that CFA alone, without the addition of BCG antigens, can activate periosteal osteogenesis. Thus, we examined the effect of CFA on bone and compared it with the effect of the vehicle alone for CFA (mineral oil, IFA).

Data concerning the effect of CFA administration on bones is conflicting. The majority of authors claim that in rats the CFA-induced arthritis is combined with severe osteolysis and an impairment of bone formation (Bonnet et al., 1993; Okazaki et al., 1998; Yoshino et al., 1998; Zhang et al., 1999). Others reported a dual activity of CFA on bone: periostitis with osteolysis at earlier stages of arthritis and periosteal new bone formation progressing to a state of near complete ankylosis at later stages (30 days post-adjuvant administration) (Jacobson et al., 1999), or endosteal osteogenesis (Tomoda et al., 1986).

Here we report an adverse effect of CFA- and IFA-induced inflammatory reactions in mice on the local

bones. While the granulomatous reaction evoked by IFA produced loss of bone or at least did not affect the net balance of bone formation and resorption, the arthritis induced by CFA had, conversely, a stimulatory effect on the local bones, as evidenced by the net bone mass increment (ashing data), histological analysis and by bone mineral densitometry (DEXA).

Stimulation of bone cells by monocytes, a substantial component of inflammatory reactions, is well documented (Peck et al., 1985). A factor produced by monocytes that affects osteoblast activity is similar or identical to IL-1 (Gowen et al., 1985) and may be important in the coupling action between osteoclasts and osteoblasts. Interleukin-1, the major inflammatory cytokine, has been shown to be capable of stimulating PGE_2 production by rat osteoblastic cells (Tatakis et al., 1988). PGE_2 has been demonstrated to add bone to all bone envelopes (Drvaric et al., 1989; Moris et al., 1990; Jee and Ma, 1997).

In the present study we found that CFA-induced arthritis can be obtained in mice and that in contrast to rats, the granulomatous reaction in mice is characterized by domination of the bone forming phase over the bone resorption phase. Whether the depletion of lymph node mast cells is in any way related to the lack of stimulation of bone formation in the IFA-treated mice remains to be elucidated. As the IFA-induced inflammation is associated with domination of bone resorption, we suggest that the *Mycobacterium tuberculosis* present in the CFA is responsible for osteoblast activation. Cell wall components of bacteria activate bone cells involved in bone remodelling (Włodarski and Galus, 1992; Blanque et al., 1998) and may reactivate adjuvant-induced arthritis in mice (Yoshino and Ohsawa, 2000). Tomoda et al. observed endosteal new bone formation and resorption in long bones of CFA-treated rats 64 days after the treatment. In our murine model system periosteal bone formation was a much earlier event than in rats.

Data concerning the effect of IFA administration on bones is scarce. In rats the application of IFA induces arthritis and together with this destruction of bones (Kleinau et al., 1994). The development of CFA-induced arthritis in rats is prevented by IFA injected 3–4 weeks before CFA challenge (Zhang et al., 1999), most likely by deviation of the T-helper cell balance of the immune response. We report here that in mice the arthritis induced by IFA is characterized by bone mineral loss. The histological and quantitative evaluation of bone involvement in the CFA- and IFA-induced arthritis in mice is consistent with the BMD scan analysis (DEXA). In the IFA-treated mice the ratio of treated to contralateral control BMD was below 1.0, which indicated a loss of or no change in the bone mass of IFA-treated limbs. When arthritis was evoked by CFA, the ratio of BMC of adjuvant-treated against contralateral control was above 1.0, demonstrating an increase in bone mass in the CFA-exposed limbs. We postulate that the BMC of murine limbs could provide a useful method for evaluation of the bone status in the course of adjuvant-induced arthritis.

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