Review

Activator protein 1 (Fos/Jun) functions in inflammatory bone and skin disease

Rainer Zenz1,2, Robert Eferl1,2, Clemens Scheinecker3, Kurt Redlich3, Josef Smolen3, Helia B Schonthaler4, Lukas Kenner1,2,5, Erwin Tschachler6 and Erwin F Wagner4†

1Ludwig Boltzmann Institute for Cancer Research, Währinger Strasse 13a, A-1090 Vienna, Austria
2Center for Biomolecular Medicine and Pharmacy, Medical University of Vienna, Währinger Strasse 13a, A-1090 Vienna, Austria
3Division of Rheumatology, Medical University of Vienna, Währinger Gürtel 18–20, A-1090 Vienna, Austria
4Research Institute of Molecular Pathology, Dr. Bohr-Gasse 7, A-1030 Vienna, Austria
5Clinical Institute of Pathology, Medical University of Vienna, Währinger Gürtel 18–20, A-1090 Vienna, Austria
6Department of Dermatology, Medical University of Vienna, Währinger Gürtel 18–20, A-1090 Vienna, Austria
†Present address: Cancer Cell Biology Program, Spanish National Cancer Center (CNIO), Melchor Fernandez Almagro 3, E-28029 Madrid, Spain

Corresponding author: Erwin F Wagner, wagner@cnio.es

Published: 18 January 2008
This article is online at http://arthritis-research.com/content/10/1/201
© 2008 BioMed Central Ltd

Abstract

Activator protein 1 (AP-1) (Fos/Jun) is a transcriptional regulator composed of members of the Fos and Jun families of DNA binding proteins. The functions of AP-1 were initially studied in mouse development as well as in the whole organism through conventional transgenic approaches, but also by gene targeting using knockout strategies. The importance of AP-1 proteins in disease pathways including the inflammatory response became fully apparent through conditional mutagenesis in mice, in particular when employing gene inactivation in a tissue-specific and inducible fashion. Besides the well-documented roles of Fos and Jun proteins in oncogenesis, where these genes can function both as tumor promoters or tumor suppressors, AP-1 proteins are being recognized as regulators of bone and immune cells, a research area termed osteoimmunology. In the present article, we review recent data regarding the functions of AP-1 as a regulator of cytokine expression and an important modulator in inflammatory diseases such as rheumatoid arthritis, psoriasis and psoriatic arthritis. These new data provide a better molecular understanding of disease pathways and should pave the road for the discovery of new targets for therapeutic applications.

Introduction

The transcription factor activator protein 1 (AP-1) consists of dimers composed of members of the Jun, Fos and activating transcription factor protein families. In contrast to the Fos proteins (Fos, FosB, Fra-1 and Fra-2), which can only heterodimerize with members of the Jun family, Jun family members (Jun, JunB and JunD) can homodimerize and heterodimerize with Fos members [1]. In addition, some members of the activating transcription factor and cAMP response element-binding protein families also dimerize with the core members of the AP-1 family to regulate a broad variety of genes [2] by binding to their promoter and enhancer regions (Figure 1).

Although members of the Jun and Fos families share a high degree of structural homology, the individual AP-1 dimers exert significant differences in their DNA binding affinity and their capability to activate or suppress gene expression [3]. AP-1 converts extracellular signals of evolutionary conserved signaling pathways like mitogen-activated protein kinase, transforming growth factor beta and Wnt into changes in the expression of specific target genes that harbor AP-1 binding sites. Growth factors, neurotransmitters, polypeptide hormones, bacterial and viral infections as well as a variety of physical and chemical stresses employ AP-1 to translate external stimuli both into short-term and long-term changes of gene expression. These stimuli activate mitogen-activated protein kinase cascades that enhance AP-1 activity; for example, through phosphorylation of distinct substrates [4].

Activator protein 1 functions in mice

Many important insights regarding the specific functions of AP-1 proteins in development and disease have been obtained from genetically modified mice and the cells derived thereof (Table 1) [1,2]. In the following sections we shall present an overview of the different phenotypes obtained from gain-of-function and loss-of-function experiments, and we shall emphasize the lessons learned from these studies.
A role for Jun/AP-1 in the control of cell proliferation has been proposed based on observations that AP-1 activity is induced upon mitogenic stimulation. Jun was shown to be primarily a positive regulator of cell proliferation. Jun-deficient fibroblasts have a marked proliferation defect in vitro, and proliferation of Jun-deficient hepatocytes was severely impaired during liver regeneration in vivo. Using conditional knockout techniques, we have recently shown that Jun/AP-1 regulates liver regeneration after partial hepatectomy through a novel molecular pathway that involves p53, p21 and the stress kinase p38\(\alpha\) [8]. Jun proteins need to be activated by Jun-amino-terminal kinases (JNKs) to fully promote cell-cycle progression. Once activated, Jun/AP-1 complexes induce the transcription of positive regulators of cell-cycle progression, such as cyclin D1, or repress negative regulators, such as the tumor suppressor p53 and the cyclin-dependent kinase inhibitor p16\(^{INK4A}\).

The expression pattern of Fos protein during embryonic mouse development indicated a possible role for the protein in endochondral ossification. Transgenic expression of Fos in many different cell types specifically affected the skeleton. In addition, chimeric mice obtained from Fos-overexpressing embryonic stem cells developed chondrogenic tumors, and ectopic expression of Fos from a ubiquitous promoter in transgenic mice resulted in the transformation of osteoblasts, leading to osteosarcomas [2]. Mice lacking Fos are viable and fertile but lack osteoclasts, resulting in an osteopetrotic phenotype [2] and references cited therein).

Transgenic mice overexpressing \(\Delta\text{FosB}\), an isoform of FosB, in osteoblasts, developed osteosclerosis with increased bone formation of the entire skeleton [11]. This phenotype is cell autonomous and is probably caused by enhanced differentiation and activity of osteoblasts. A similar osteosclerotic phenotype was observed in transgenic mice expressing Fra-1 in osteoblasts [12]. Ablation of Fra-1 during development resulted in lethality around embryonic day 10 due to placental defects, thereby preventing the analysis Fra-1 function in later development [2]. Applying conditional knockout techniques, we were recently able to demonstrate that mice lacking Fra-1 are viable and fertile but developed osteopenia, a low bone mass disease. Conditional Fra-1 knockout mice appeared to have normal numbers of osteoblasts and osteoclasts, but expressed reduced amounts of bone matrix components such as osteocalcin, collagen 1a2 and matrix Gla protein that are produced by osteoblasts and chondrocytes [13]. We

The activator protein 1 transcription factor. The dimeric activator protein 1 (AP-1) transcription factor is composed of Jun and Fos proteins. Jun proteins form homodimers or heterodimers with Fos proteins through their leucine-zipper domains. The different dimer combinations recognize different sequence elements in the promoters and enhancers of target genes. Only the classic TPA-responsive element with the consensus sequence TGACTCA is shown. The AP-1 dimers recognize the specific response elements via the basic domain that is adjacent to the leucine-zipper domain and represent an \(\alpha\)-helical structure. Among the target genes of AP-1 are important regulators of cell proliferation, differentiation and apoptosis. Some AP-1 targets are positively regulated (+), negatively regulated (–), or positively and negatively regulated (+/–) depending on the AP-1 dimer composition.

**Multiple roles of Jun proteins**

Transgenic expression of Jun, JunD or JunB in transgenic mice did not result in an overt phenotype, although targeted overexpression of JunB in T lymphocytes interfered with the differentiation of T helper cells ([1] and references cited therein), implying a role of JunB in T cell development. Ectopic expression of JunD under the control of the ubiquitin C promoter caused a reduction in the number of peripheral T cells and B cells, further suggesting a role of JunD in the regulation of the immune system ([1] and references cited therein). Jun was recently identified as a regulator of \(\alpha\)\(/\gamma\)\(\delta\) T-cell development by repressing IL7R\(\alpha\) expression, which is essential for the \(\gamma\)\(\delta\) lineage decision [5].

Jun and JunB are essential proteins for embryonic development, whereas JunD is required postnatally. Fetuses lacking Jun die between embryonic day 12.5 and embryonic day 14.5 of development with defects in liver development and heart morphogenesis [1]. Embryos lacking JunB show impaired vasculogenesis and angiogenesis in the extraembryonal tissue, leading to embryonic lethality around embryonic day 9.5 [6]. In contrast, mice lacking JunD are viable but exhibit reduced postnatal growth and multiple age-dependent defects in reproduction, hormone imbalance and impaired spermatogenesis [7].
therefore speculate that Fra-1 functions in bone forming osteoblasts, mainly by affecting the activity of the cells through the regulation of matrix production and not by affecting the proliferation or differentiation of cells.

Mice overexpressing Fra-2 under the control of a cytomegalovirus promoter were reported to display ocular malformations due to disrupted development of anterior eye structures [14]. When Fra-2 was broadly expressed from the H2 promoter in many organs, however, the mice developed a severe fibrotic disease mostly in the lung, as well as occasional fibrosarcomas, alongside an increased bone mass (A Bozec, R Eferl, P Hasselblatt, unpublished data). In contrast, the absence of Fra-2 in embryos and newborn mice affected hypertrophic chondrocyte differentiation and matrix production [15], and mutant pups died shortly after birth [16]. Moreover, Fra-2 knockout newborns exhibited cell autonomous defects in osteoclasts and osteoblasts that were dependent on signaling from the LIF/LIF-receptor system (A Bozec, L Bakiri, unpublished data). Chondrocyte-specific

<table>
<thead>
<tr>
<th>Activator protein 1 protein</th>
<th>Phenotype</th>
<th>Affected organs/cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2Kb-Jun</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Ubiquitin C-JunB*</td>
<td>Increased bone mass</td>
<td>Not defined</td>
</tr>
<tr>
<td>CD4-JunB</td>
<td>Enhanced T helper cell 2 maturation</td>
<td>Thymus, CD4 thymocytes</td>
</tr>
<tr>
<td>Ubiquitin C-JunD</td>
<td>Peripheral T cells and B cells reduced</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>H2Kb-Fos</td>
<td>Osteosarcoma</td>
<td>Bone, osteoblasts</td>
</tr>
<tr>
<td>H2Kb-Fos/Rsk-2−/−</td>
<td>Reduced osteosarcoma</td>
<td>Bone, osteoblasts</td>
</tr>
<tr>
<td>H2Kb-FosB</td>
<td>None</td>
<td>Bone</td>
</tr>
<tr>
<td>TCRβ-ΔFosB</td>
<td>Impaired T cell differentiation</td>
<td>Thymus, immature thymocytes</td>
</tr>
<tr>
<td>NSE-ΔFosB</td>
<td>Osteosclerosis</td>
<td>Bone, osteoblasts</td>
</tr>
<tr>
<td>H2Kb-Fra-1</td>
<td>Osteosclerosis</td>
<td>Bone, osteoblasts</td>
</tr>
<tr>
<td>CMV-Fra-2</td>
<td>Ocular malformations</td>
<td>Anterior eye structure</td>
</tr>
<tr>
<td>H2Kb-Fra-2−/−</td>
<td>Increased bone mass, fibrosis</td>
<td>Bone, internal organs, skin</td>
</tr>
</tbody>
</table>

Knockout

<table>
<thead>
<tr>
<th>Activator protein 1 protein</th>
<th>Phenotype</th>
<th>Affected organs/cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jun</td>
<td>Embryonic lethal on embryonic day 12.5</td>
<td>Liver, heart, neural crest</td>
</tr>
<tr>
<td>JunB</td>
<td>Embryonic lethal on embryonic day 10</td>
<td>Extraembryonic tissues</td>
</tr>
<tr>
<td>JunD</td>
<td>Male sterility</td>
<td>Testis, spermatides</td>
</tr>
<tr>
<td>c-Fos</td>
<td>Osteopetrosis</td>
<td>Bone, osteoclasts</td>
</tr>
<tr>
<td>FosB</td>
<td>Nurturing defect</td>
<td>Brain, hypothalamus</td>
</tr>
<tr>
<td>Fra-1</td>
<td>Embryonic lethal on embryonic day 9.5</td>
<td>Extraembryonic tissue</td>
</tr>
<tr>
<td>Fra-2</td>
<td>Lethal at birth</td>
<td>Bone, osteoclasts</td>
</tr>
</tbody>
</table>

Conditional

<table>
<thead>
<tr>
<th>Activator protein 1 protein</th>
<th>Phenotype</th>
<th>Affected organs/cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfp-cre Jun</td>
<td>Liver regeneration defect</td>
<td>Liver, hepatocytes</td>
</tr>
<tr>
<td>Col2a1-cre Jun</td>
<td>Scoliosis</td>
<td>Bone, notochordal cells</td>
</tr>
<tr>
<td>Nestin-cre Jun</td>
<td>Axonal regeneration defect</td>
<td>Central nervous system, motoneurons</td>
</tr>
<tr>
<td>MORE-cre JunB</td>
<td>Osteopenia</td>
<td>Bone, osteoclasts, osteoblasts</td>
</tr>
<tr>
<td>K5-cre Jun</td>
<td>Eyes open at birth, reduced skin tumors</td>
<td>Keratinocytes</td>
</tr>
<tr>
<td>Nestin-cre Fos</td>
<td>Learning defects</td>
<td>Brain, hippocampal neurons</td>
</tr>
<tr>
<td>MORE-cre Fra-1</td>
<td>Osteopenia</td>
<td>Bone, osteoblasts</td>
</tr>
</tbody>
</table>

Inducible

<table>
<thead>
<tr>
<th>Activator protein 1 protein</th>
<th>Phenotype</th>
<th>Affected organs/cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>K5-creER&lt;sup&gt;+&lt;/sup&gt;JunB + Jun</td>
<td>Psoriasis-like disease</td>
<td>Skin, joints, keratinocytes</td>
</tr>
</tbody>
</table>

Knockout, conditional knockout and gain of function (transgenic) approaches applied to study the role of Jun and Fos proteins during development and in diseases. The gain-of-function approaches were performed with different promoters, either leading to ubiquitous expression (for example, H2Kb, ubiquitin C, or cytomegalovirus (CMV)) or to tissue-specific expression (for example, CD4, TCRβ), or neuron-specific enolase (NSE) of the transgenes. *Unpublished data from the Wagner Laboratory.*
inactivation of Fra-2 led to cell autonomous defects in cartilage, since mutant mice were growth retarded and developed a kyphosis-like phenotype [15]. Interestingly, mice lacking JunB are also osteopenic due to cell-autonomous osteoblast and osteoclast defects [17].

Taken together, Fos/AP-1 proteins are important regulators of bone formation, and therapeutic interventions acting on AP-1 signaling might provide a powerful approach for the treatment of low bone mass diseases.

**Activator protein 1 in inflammation**

Chronic inflammatory diseases, such as inflammatory bowel disease, chronic obstructive pulmonary disease, rheumatoid arthritis (RA), psoriasis and psoriatic arthritis, are affecting a large segment of the population. In addition, cancer and even metabolic diseases, such as type 2 diabetes or atherosclerosis, are believed to have an inflammatory component [18]. It is thought that in several of these diseases chemotactic/chemoattractant proteins and cytokines are released at the side of injury or infection, which then attracts innate and adaptive immune cells. The cytokine milieu together with the immune cells triggers a cascade of events, called the inflammatory process. Interestingly, many cytokine genes are regulated cooperatively by a transcription factor complex consisting of AP-1 and nuclear factor of activated T cells (NFAT). NFAT-dependent gene regulation has been demonstrated for IL-2, IL-3, granulocyte–macrophage colony-stimulating factor, IL-4, IL-5, IL-13, IFNγ, TNFα, CD40L, FasL, CD5, Igκ, CD25 and the chemokines IL-8 and MIP1κ. Importantly, for the majority of these genes, the induction with AP-1 appears essential.

The innate immune system employs cellular components such as macrophages or dendritic cells and humoral components of the complement system to respond to infectious agents. The activation of Toll-like receptors is an important starting point for the activation of innate immunity. Once activated, Toll-like receptors lead among other events to the differentiation of macrophages and to the production of several cytokines such as TNFα, IL-1, IL-6 or IL-12. The signaling of Toll-like receptors leading to cytokine production is integrated by adapter molecules such as MyD88 and TRAF6 that eventually activate NF-κB and AP-1 [19].

Allergic asthma, RA and psoriasis are thought to be inflammatory diseases mediated by activated T cells. AP-1 has been shown to be involved in the differentiation of naïve T cells into T helper 1 cells and T helper 2 cells, which is a hallmark of the T cell-dependent immune response. JunB positively regulates IL-4 expression and accumulates in T helper 2 cells during differentiation [20]. In agreement, loss of JunB in polarized T helper 2 cells in vitro is followed by deregulated expression of T-helper-2-specific cytokines and by expression of IFNγ and T-bet, which are known as key regulators of T helper 1 cells [21]. The molecular mecha-

Glucocorticoids are very effective in controlling inflammation and are used for the treatment of autoimmune diseases such as RA. Expression of several cytokines such as IL-1, IL-2 or IFNγ is activated by AP-1 and other transcription factors, but is repressed by the glucocorticoid receptor (GR). Recent data suggest that the GR prevents the interaction between DNA-bound AP-1 complexes and transcriptional coactivators. Irrespective of the exact mechanism, the ability of the GR to repress the proinflammatory transcription factors AP-1 and NF-κB seems the most important function of the GR. This has been demonstrated with genetically modified GRdim/dim mice, whose GR is unable to bind to GR-responsive DNA elements but is still capable of transrepressing AP-1 and NF-κB [24].

**Functions of activator protein 1 in the pathogenesis of inflammatory bone diseases**

Bone is a highly dynamic organ that is continuously re-modeled by osteoclasts and osteoblasts. Any disturbance in the balance between these cells causes a pathogenic change in bone mass. This could either be a loss of bone mass as observed in postmenopausal osteoporosis or a gain of bone mass as observed in osteopetrosis. Evidence from a variety of mouse models suggests that the AP-1 transcription factor is directly or indirectly implicated in the development of several bone diseases [2]. AP-1 influences the pathogenic outcome of bone diseases not only via differentiation of bone cells but also via inflammatory processes. We shall focus on two types of inflammatory diseases, RA and psoriatic arthritis, and shall discuss the potential role of the AP-1 transcription factor.

**Rheumatoid arthritis and activator protein 1**

RA is considered an autoimmune disorder where the immune system preferentially attacks the joints. Extraarticular tissues such as skin, blood vessels, the heart, the lungs and muscles, however, can also be affected in a systemic manner. Besides aging, several risk factors have been identified, such as gender, environmental conditions and genetic predisposition. In addition, a strong genetic association between the major histocompatibility complex antigen DR4 and the prevalence for RA has been observed [25].

Histopathologically, RA is characterized by synovial inflammation, cartilage destruction and erosion of subchondral
Activated T cells are considered the major inflammatory component that affects the severity of RA [26]; however, others see cells of the monocyte/macrophage lineage or synovial fibroblasts as the main culprits [27]. The cellular mechanism by which T cells promote joint destruction in RA has been unravelled using different animal models. For example, collagen-induced arthritis has been widely used as an animal model for RA. The disease is induced by immunization of mice or rats with type II collagen and an adjuvant.

RA is also characterized by the overexpression of pro-inflammatory cytokines. In fact, a particularly important genetic model that was used to investigate the cellular interactions in RA is transgenic mice expressing human TNFα from a globin promoter (hTNFtg mice). The hTNFtg mice develop a RA-like disease that is characterized by inflammation of the joints, joint swelling and bone erosions (see later Figure 4e,f). Breeding of hTNFtg mice with knockout mice lacking the AP-1 component Fos, and therefore devoid of osteoclasts, demonstrated the essential requirement for osteoclasts in RA. hTNFtg Fos−/− mice are completely protected from hTNFtg-induced bone erosion, although the severity of synovial inflammation as well as paw swelling and the reduction of grip strength were not ameliorated. Similar studies where osteoprotegerin was used to inhibit osteoclast differentiation suggest that activated cells present in the rheumatoid synovial membrane, such as T cells or fibroblasts, promote Fos-dependent differentiation of macrophage precursors into osteoclasts, thereby promoting bone resorption [28].

One key signaling molecule that was initially identified on activated T cells and as a regulator of T cell function is the receptor activator of NF-κB ligand (RANKL) – also called TRANCE, ODF, OPGL or TNFSF11 [29]. Under pathogenic conditions such as RA, RANKL is also secreted by a variety of synovial cells including inflammatory T cells, thereby promoting extensive osteoclastogenesis and bone resorption [30]. One potent negative regulator of RANKL is the decoy receptor osteoprotegerin, which competes with RANKL for binding to the receptor activator of NF-κB receptor on osteoclast precursors, thereby inhibiting RANKL-induced osteoclastogenesis [31]. In RA, however, the ratio between RANKL and osteoprotegerin is shifted in favor of RANKL, resulting in a net increase of osteoclastogenesis. Based on this knowledge, a human anti-RANKL antibody called Denosumab has been developed and is currently being tested for treatment of postmenopausal osteoporosis as well as of local bone erosions in RA [32].

The most important transcription factor complexes that are activated by RANKL/TRA signals are NF-κB and Fos/AP-1 [2]. The inactivation of NF-κB or Fos causes severe osteoporosis due to the lack of osteoclasts. Two key target genes of Fos in osteoclastogenesis have been identified recently. The first gene, NFATc1, turned out to be a promoter of osteoclastogenesis, whereas the second gene, IFNβ, is an antagonist. NFATc1 is not solely a downstream target of Fos but also cooperates with Fos and Jun proteins to induce osteoclast-specific genes such as tartrate-resistant acid phosphatase or cathepsin K. Most importantly, ectopic expression of NFATc1 can rescue the osteoclast differentiation defect of Fos-deficient macrocyte precursors, suggesting it is the most critical target gene of Fos in osteoclastogenesis [33]. The other Fos target gene that is activated by RANKL is IFNβ. Surprisingly, IFNβ has been shown to reduce the expression of Fos in osteoclast precursors. This has led to a model where IFNβ provides a negative feedback loop that prevents extensive osteoclastogenic activity of Fos [34]. The implication of NFATc1 and IFNβ in RA is very likely, since these proteins are key target genes of Fos. Further studies are required, however, before their potential use as therapeutic targets is taken into account.

AP-1 activity can also affect the severity of RA at a level different from osteoclastogenesis. In addition to osteoclast-mediated bone erosion, several molecules are secreted by synovial fibroblasts that contribute to matrix degradation. Of particular importance are matrix metalloproteinases (MMPs) that are regulated by AP-1 and degrade collagen, fibronectin or other components of the extracellular matrix. The major MMPs that are implicated in RA are MMP-1, MMP-9, MMP-13 and MMP-14 (MT1-MMP) [35]. These MMPs are expressed by activated osteoclasts or by synovial fibroblasts, or by both. The significance of AP-1-mediated MMP regulation in RA, however, has not yet been demonstrated in suitable mouse models.

Signals that lead to activation of Jun have been implicated in RA. In particular, JNK is highly activated in synovial fibroblasts of RA. The use of the JNK inhibitor SP600125 blocked accumulation of phospho-Jun in synovial fibroblasts, reduced the expression of the Jun target gene collagenase-3 and ameliorated bone erosion after collagen-induced arthritis in rats [36]. JNK/Jun signaling should therefore also be considered a potential therapeutic target for RA.

In summary, AP-1 activity is induced in RA by inflammatory cytokines and has a complex impact on osteoclast differentiation and production of soluble mediators of bone erosion. It can be anticipated that several AP-1 components or signaling pathways leading to AP-1 activation may provide valuable drug targets for therapy of RA in the future. At present, however, therapies that target TNF-α, IL-1, IL-6, B cell and T cell costimulation are the most effective biological treatments [37].

**Activator protein 1 and epidermal disease**

AP-1 has been proposed to play important functions in the epidermis of the skin, from differentiation to wound repair and carcinogenesis. Conditional, epidermis-specific knockout
mice recently provided insights into the function of Jun/AP-1
in skin biology in vivo [1]. Jun is regarded a positive regulator
of keratinocyte proliferation/differentiation through its direct
transcriptional effect on epidermal growth factor receptor
expression [38]. In contrast, JunB can antagonize the prolif-
eration of keratinocytes and hematopoietic stem cells. Adult
mice lacking JunB in the epidermis developed skin
ulcerations in the facial area, hypergranulopoiesis and lost
bone mass, most probably mediated by granulocyte colony-
stimulating factor release from the epidermis (A Meixner, R
Zenz, HB Schonthaler, L Kenner, H Scheuch, JM Penninger,
EF Wagner, manuscript under revision). Epidermis-specific
deletion of JunB therefore appears to affect distant organs
affecting myelopoiesis and bone homeostasis, supporting the
notion of an endocrine-like function of the skin.

Psoriasis and the activator-protein-1-dependent mouse
model
Psoriasis is a chronic inflammatory skin disease affecting
1–3% of the general population. At the histopathological
level the disease is characterized by accelerated proliferation
and altered differentiation of keratinocytes and extensive
mixed leukocyte infiltrates consisting of T cells, monocytes
and neutrophils [39]. In up to 40% of patients, the skin
disease is associated with arthritis [40]. The beneficial
therapeutic effects of immunosuppressive drugs such as
cyclosporine as well as the new class of ‘biological agents’
have established a central role of immune cells in the
pathogenesis of psoriasis [39]. It is still controversial,
however, whether the involvement of immune cells is the
cause of or the consequence of the psoriasis phenotype
observed in keratinocytes [41]. Although at least six different
psoriasis susceptibility loci (PSORS1–PSORS6) have been
mapped in the human genome, the genetic basis of psoriasis
remains largely unknown [42].

We recently described that expression of human JunB, which
is localized in the PSORS6 locus (psoriasis susceptibility
locus 6; 19p13), was reduced in lesional areas of severe
psoriasis, suggesting a possible role of JunB in the develop-
ment of the disease [43]. Moreover, reduced AP-1 binding
activity was also reported in lesional skin from psoriatic
patients [44]. In contrast, others reported a slight but
insignificant increase of JunB mRNA and protein expression
in psoriasis vulgaris lesions [45], and argued that induced
JunB expression in keratinocytes may be part of an overall
inflammatory response. We recently found that there is a
heterogeneity in the expression of JunB within lesional skin
(Figure 2), but JunB expression also seems to be variable
between individuals in nonlesional skin. It is presently unclear
whether these differences in gene expression are caused by
the heterogeneity and complexity of the disease. Additional
experiments with human samples as well as human keratino-
cyte cultures are necessary to establish the role of JunB in
skin inflammation and whether modulation of JunB expression
is associated with the pathogenesis of the disease.

To downregulate Jun/AP-1 expression in the epidermis of
adult mice, we generated epidermis-specific, inducible single-
knockout and double-knockout mice for JunB and Jun
(Figure 3a). Mice harboring conditional JunB and Jun alleles
were crossed to K5-Cre-ER\(^{T}\) transgenic mice, in which
tamoxifen efficiently induced Cre-mediated deletion of JunB
and/or Jun in the basal layer of the epidermis. Adult single-
mutant and double-mutant mice and their littermate controls
were injected with tamoxifen and monitored for 14 days
(Figure 3b). Inducible deletion of JunB or Jun in the epidermis
revealed no signs of a skin phenotype up to 2 months after
deletion. Interestingly, JunB/Jun double-mutant mice deve-
loped skin alterations mainly affecting hairless skin, which
resemble lesions observed in patients with psoriasis. One
hundred percent of the double-mutant mice showed a strong
phenotype with inflamed scaly plaques affecting primarily the
ears, paws and tail, and less frequently the hairy back skin
after 3 weeks (Figure 3c–h). The affected skin of double-
mutant mice showed the hallmarks of psoriasis, with a
strongly thickened epidermis, hyperkeratosis (thickened
keratinized upper layers) with nucleated keratinocytes in the
cornified layer (parakeratosis) and increased subepidermal
vascularization (Figure 3e,f). Intraepidermal T cells, epidermal

Figure 2

Heterogeneous JunB expression within lesional psoriatic skin. Immune reactivity of a monoclonal antibody against JunB within a psoriatic lesion.
(a) Distinct anti-JunB reactivity of a parakeratotic lesion. JunB expression is observed throughout all epidermal layers (left side, arrow), whereas it is reduced on the right side of the lesion (see arrow). (b) A different area of the same lesion. A virtual absence of nuclear reactivity is seen in basal keratinocytes, whereas strong nuclear activity is detected in the upper suprabasal epidermal layers ((a) and (b) arrows).
microabscesses and the typical inflammatory cell infiltrate consisting of neutrophils were seen together with increased numbers of macrophages in the dermis. Arthropathic lesions seen in 5–40% of psoriasis patients were observed in double-mutant mice with inflammatory infiltrates in the joint regions along with bone destruction and periostitis (see below) [43].

Since many of the histological and molecular hallmarks of psoriasis are reproduced in mice with epidermal deletion of JunB and Jun, we employed this mouse model to address the role of immunocytes during disease development. JunB and Jun were therefore deleted in mice deficient for Rag2 that lack functional T cells and B cells. Interestingly, the skin phenotype of Rag2-deficient JunB/Jun double-mutant mice was milder but still present when compared with JunB/Jun double-mutant mice, suggesting a minor role for T cells and B cells in the etiology of the skin disease in this model. Arthritic-like lesions were almost absent in these mice, however, strongly implicating the involvement of T cells in the development of the phenotype [43]. It will be interesting to analyze in detail the immunocyte subsets to further explore the role of macrophages and dendritic cells. Both cell types might contribute to the production of TNFα, which was still highly expressed in the epidermis even in the absence of functional T cells.

Recently developed biological agents are directed towards inhibiting TNFα signaling. We therefore genetically deleted JunB and Jun in TNFR1 knockout mice. Interestingly, this deletion did not prevent the development of the skin phenotype, although histological analyses showed a milder phenotype when compared with JunB/Jun double-mutant mice. The inflammation of the joint regions was again almost absent, demonstrating a functional contribution of TNFα signaling via TNFR1 to the etiology of the joint lesions.

Another key finding in double-mutant mice was the rapid upregulation of genes encoding the Ca²⁺-binding proteins S100a8 and S100a9 in keratinocytes upon deletion of JunB and Jun, both in vivo and in vitro. The S100a8 and S100a9 genes map to the PSORS4 region and have been found strongly upregulated in affected areas of psoriatic skin. The S100a8/S100a9 complex functions as a chemotactic signal for T cells and neutrophils. S100a9 knockout mice are viable and fertile but do not form S100a8/S100a9 complexes [46]. These mice are currently employed in our laboratory to test the functional contribution of S100a8/S100a9 in disease development. Preliminary results suggest that S100a8/S100a9 may indeed be an important signal early in the development of the phenotype, since the disease phenotype appears to be altered in mice lacking S100a9 (HB Schönthaler, EF Wagner, unpublished data).

The mouse model lacking JunB and Jun in the epidermis largely recapitulates the histological and molecular hallmarks of psoriasis and the typical inflammatory cell infiltrate consisting of neutrophils were seen together with increased numbers of macrophages in the dermis. Arthropathic lesions seen in 5–40% of psoriasis patients were observed in double-mutant mice with inflammatory infiltrates in the joint regions along with bone destruction and periostitis (see below) [43].

Recently developed biological agents are directed towards inhibiting TNFα signaling. We therefore genetically deleted JunB and Jun in TNFR1 knockout mice. Interestingly, this deletion did not prevent the development of the skin phenotype, although histological analyses showed a milder phenotype when compared with JunB/Jun double-mutant mice. The inflammation of the joint regions was again almost absent, demonstrating a functional contribution of TNFα signaling via TNFR1 to the etiology of the joint lesions.

Another key finding in double-mutant mice was the rapid upregulation of genes encoding the Ca²⁺-binding proteins S100a8 and S100a9 in keratinocytes upon deletion of JunB and Jun, both in vivo and in vitro. The S100a8 and S100a9 genes map to the PSORS4 region and have been found strongly upregulated in affected areas of psoriatic skin. The S100a8/S100a9 complex functions as a chemotactic signal for T cells and neutrophils. S100a9 knockout mice are viable and fertile but do not form S100a8/S100a9 complexes [46]. These mice are currently employed in our laboratory to test the functional contribution of S100a8/S100a9 in disease development. Preliminary results suggest that S100a8/S100a9 may indeed be an important signal early in the development of the phenotype, since the disease phenotype appears to be altered in mice lacking S100a9 (HB Schönthaler, EF Wagner, unpublished data).

The mouse model lacking JunB and Jun in the epidermis largely recapitulates the histological and molecular hallmarks of psoriasis and the typical inflammatory cell infiltrate consisting of neutrophils were seen together with increased numbers of macrophages in the dermis. Arthropathic lesions seen in 5–40% of psoriasis patients were observed in double-mutant mice with inflammatory infiltrates in the joint regions along with bone destruction and periostitis (see below) [43].

Since many of the histological and molecular hallmarks of psoriasis are reproduced in mice with epidermal deletion of JunB and Jun, we employed this mouse model to address the role of immunocytes during disease development. JunB and Jun were therefore deleted in mice deficient for Rag2 that lack functional T cells and B cells. Interestingly, the skin phenotype of Rag2-deficient JunB/Jun double-mutant mice was milder but still present when compared with JunB/Jun double-mutant mice, suggesting a minor role for T cells and B cells in the etiology of the skin disease in this model. Arthritic-like lesions were almost absent in these mice, however, strongly implicating the involvement of T cells in the development of the phenotype [43]. It will be interesting to analyze in detail the immunocyte subsets to further explore the role of macrophages and dendritic cells. Both cell types might contribute to the production of TNFα, which was still highly expressed in the epidermis even in the absence of functional T cells.

Recently developed biological agents are directed towards inhibiting TNFα signaling. We therefore genetically deleted JunB and Jun in TNFR1 knockout mice. Interestingly, this deletion did not prevent the development of the skin phenotype, although histological analyses showed a milder phenotype when compared with JunB/Jun double-mutant mice. The inflammation of the joint regions was again almost absent, demonstrating a functional contribution of TNFα signaling via TNFR1 to the etiology of the joint lesions.

Another key finding in double-mutant mice was the rapid upregulation of genes encoding the Ca²⁺-binding proteins S100a8 and S100a9 in keratinocytes upon deletion of JunB and Jun, both in vivo and in vitro. The S100a8 and S100a9 genes map to the PSORS4 region and have been found strongly upregulated in affected areas of psoriatic skin. The S100a8/S100a9 complex functions as a chemotactic signal for T cells and neutrophils. S100a9 knockout mice are viable and fertile but do not form S100a8/S100a9 complexes [46]. These mice are currently employed in our laboratory to test the functional contribution of S100a8/S100a9 in disease development. Preliminary results suggest that S100a8/S100a9 may indeed be an important signal early in the development of the phenotype, since the disease phenotype appears to be altered in mice lacking S100a9 (HB Schönthaler, EF Wagner, unpublished data).

The mouse model lacking JunB and Jun in the epidermis largely recapitulates the histological and molecular hallmarks of psoriasis and the typical inflammatory cell infiltrate consisting of neutrophils were seen together with increased numbers of macrophages in the dermis. Arthropathic lesions seen in 5–40% of psoriasis patients were observed in double-mutant mice with inflammatory infiltrates in the joint regions along with bone destruction and periostitis (see below) [43].
seen in psoriasis. Previous attempts to reproduce the psoriatic phenotype by expression of inflammatory mediators or growth factors such as TNFα, IL-1β, IFNγ, keratinocyte growth factor, vascular endothelial growth factor, transforming growth factor beta 1, Stat3 and others (reviewed in [47]) yielded also phenotypes partially resembling psoriasis. Moreover, almost all of the mouse models discussed above showed no arthritic lesions [48].

Psoriasis-like arthropathy in the inducible, epidermis-specific Jun mouse model
The psoriasis-like disease in JunB/Jun double-mutant mice is characterized by periarticular inflammation with an asymmetric pattern of involvement. The first clinical signs of the disease are an elevation and thickening of the nails accompanied by sausage-like swelling of one or more toes, which are not always uniformly affected (Figure 3h). Different manifestations of the disease, such as synovitis, dactylitis and enthesitis – all of which occur rapidly – were recognized by microscopic analysis (L. Kenner, unpublished data). Moreover, the severe form of the disease involved individual toes with shortening and thickening of the distal phalanx covered by hyperkeratotic, edematous skin. Distal interphalangeal joints and cartilage were only mildly affected. The ‘sausage’ digit was characterized by extensive subcutaneous edema accompanied by a proliferation of small blood vessels and an
acute inflammatory reaction involving numerous neutrophils. Inflammatory infiltrates were observed within the proliferating and thickened synovial lining layer with profound lymphocytic and granulocyte infiltration as well as the presence of small vessels (L. Kenner, unpublished data). Tenosynovitis with perimyscular and tendon sheath edema as well as cell infiltrations were also seen. The proliferative periostitis affected both the underlying bone and the overlying nail base in a continuous process. The overlying dermis appeared also with a mixed infiltrate, since the dermis was edematous and hyperplastic.

These changes described above are reminiscent in their severity to inflammatory skin infiltrates. In advanced stages, dactylitis led to an almost complete destruction of the distal phalanx (Figure 4b). Osteoclasts invading the bone were observed at the front of erosions and suggested a peristemeum-derived, sometimes granulomatous, tissue (Figure 4d). It is worth pointing out that these manifestations are different from the joint pathology observed in the hTNFtg mouse model of RA [49].

As in human RA, no destruction of the distal phalanx can be seen in hTNFtg mice and the erosive arthritis typically spares the distal interphalangeal joints (Figure 4f). Moreover, pannus formation and osteoclast-mediated subchondral bone destruction is prominent in hTNFtg mice (Figure 4f).

The histopathology of JunB/Jun double-mutant mice differs from human RA but is reminiscent of a rare form of psoriasis pustulosa called akrodermatitis continua suppurativa Hallopeau [50]. In this disease lesions typically develop on the distal portion of the digits, involve the nail bed and spread proximally with time, finally leading to onychodystrophy [51]. The relationship between skin and nail involvement and joint manifestations is not resolved [51]. A detailed analysis at different time points during disease progression starting from toe involvement until joint disease could certainly help to clarify this question.

Conclusions

AP-1 is considered a transcription factor of general importance for many cellular processes in different organs. It was therefore somewhat surprising that gene knockout experiments demonstrated rather tissue-specific and cell-specific functions of individual AP-1 components, particularly in development. Some of these specific functions from conditional AP-1 knockout studies are implicated in diseases that are linked to inflammatory processes such as RA or psoriasis. Under these circumstances, AP-1 might be implicated as a downstream mediator of cytokine signaling. Alternatively, deregulated AP-1 activity might directly be causally involved in the initiation of disease development before inflammation takes place. The latter possibility is convincingly demonstrated in the psoriasis-like mouse model with deletions of JunB and Jun in epidermal cells. Such mouse models are essential to dissect the molecular pathways that lead to various organ-specific phenotypes that can be observed in more complex diseases. These models can also be employed for preclinical studies with known or novel therapeutic drugs, and they may reveal unexpected environmental factors that have not been considered in diseases such as psoriasis. For example, we have obtained preliminary data in the psoriasis mouse model suggesting that ciprofloxacin significantly delayed the onset of the skin disease and prevented the arthritic-like phenotype. This observation implies that resident bacteria might contribute to the manifestation of the joint disease.

It is plausible that different molecular pathomechanisms are responsible for the organ-specific manifestations of complex diseases. This would imply that therapeutic strategies have to be custom-tailored for each mechanism and used in a combinatorial manner to give attribute to all disease manifestations. Alternatively, identification of factors and pathways such as AP-1 that could be directly involved in diseases such as psoriasis may offer the possibility for a target-directed therapy.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

The authors are very grateful to members of the Wagner laboratory for critical reading of the manuscript and helpful comments, and they thank Hannes Tkadletz for help in preparing the illustrations. RZ, RE, and LK are funded by the Ludwig Boltzmann Society. RE is also funded by the SFB grant SFB-F28. The Research Institute of Molecular Pathology is funded by Boehringer Ingelheim, and the present work was supported by the Austrian Industrial Research Promotion Fund.

References


