gel and transferred to nylon membranes (Hybond-N, Amersham Biosciences). Hybridization was performed as previously described[48] using [^{32}P]-labeled DNA probes made with a Ready-to-Go DNA labeling kit (Amersham Biosciences).

PCR and RT–PCR analyses

PCR amplification for screening of ES clones and for genotyping of embryonic fibroblasts, embryos and mice was performed with two pairs of primers: primer P1 (5'-CCGTATGTCTGAAGACAGCTACTGT-3' from intron 6) and primer P2 (5'-CTCTATGGCTTCTGAGGCG-GAAAG-3' from PGKneobpA) for detection of the targeted allele, and primer P3 (5'-ATGAGATCCTGTGCCTTCTTCCCG-3' from intron 6) and primer P4 (5'-GTCACCGTGGGATAGGATACAGCA-3' from exon 7) for the normal allele. PCR was carried out as follows: initial denaturation at 96°C for 3 min following by 40 cycles of 94°C for 1 min, 67°C for 2 min, 72°C for 3 min and extension at 72°C for 10 min.

For RT–PCR analysis, total RNAs (3 μg) isolated from E10.5 embryos using Isogen (Nippongene, Japan) were used for first-strand cDNA synthesis with an oligo-dT primer on Ready-To-Go RT–PCR beads (Amersham Biosciences), according to the manufacturer's recommendations. First-strand cDNAs were amplified with three sets of primers: primers P4 and P5 (5'-ATGGCGGAACTGTGTGACTCG-3' on exon 5) for detection of normal caspase-8 transcripts, primers P5 and P6 (5'-ATGTTTCAGGTTCAGGGGGAGGTG-3' in the SV40 polyA region) for truncated caspase-8 transcripts, and primers P7 (5'-CCTGATTGTTGCTGCTGGTGTTGG-3') and P8 (5'-GTCACGAA-CAGCAAAGCGACCAAG-3') for mouse EF1α transcripts as a standard control. PCR was performed under the conditions described above and PCR products were analyzed by 1% agarose gel electrophoresis.

Establishment of stable transfectants and embryonic fibroblasts

To establish transfectants stably-expressing mouse caspase-8, we used JB-6 cells, a human T lymphoma Jurkat cell variant that is caspase-8-deficient.[49] We transfected mouse caspase-8 cDNA with the neo resistance gene into this variant and selected them in 1.5 mg/ml of G418. An established JB-6 stable clone and parental JB-6 cells were maintained in RPMI-1640 with 10% fetal calf serum. MEF cells were established from E10.5 embryos and their genotype was determined by PCR. These cells were maintained in D-MEM with 10% fetal calf serum.

Immunoblot analysis

For preparation of cell lysates, stable transfectants expressing mouse caspase-8 and MEF cells were lysed in lysis buffer (150 mM NaCl, 0.1% Triton X-100, 10% glycerol, 1% EDTA, a cocktail of protease inhibitors and 20 mM Tris-HCl pH 7.5). Similarly, cell lysates from E8.5 embryos and from several E17 embryonic tissues were prepared after homogenization in the lysis buffer. These lysates were then analyzed by SDS–PAGE followed by immunoblotting with anti-mouse caspase-8 and anti-actin (Chemicon) monoclonal antibodies. Prior to immunoblotting, the membrane transferred with cell lysates of MEF cells was immersed in phosphate-buffered saline (PBS) containing 3% formaldehyde for 10 min. Then, immunoblot analysis was performed as described previously.[50] An anti-mouse caspase-8 monoclonal antibody was generated against full-length of mouse caspase-8 produced in *E. coli* and recognizes the DED domain of caspase-8 (Kazama *et al*., unpublished data).

Ex vivo whole-embryo culture

For whole-embryo culture, embryos (34–38 somites) were dissected at E10.5 from pregnant mice after heterozygous mating. Cultivation of embryos was carried out at 37°C for 24 h in rat serum containing 2 mg/ml glucose as described.[28] After culture, embryos (45–50 somites) were fixed in 4% paraformaldehyde (PFA) in PBS overnight for histology, immunohistochemistry and *in situ* hybridization. Genomic DNAs for genotyping were prepared from yolk sacs after culture.

In situ hybridization

Embryos were dissected at E10.5 and E11.5 and after whole-embryo culture, and fixed with 4% PFA in PBS overnight. Frozen sections were cut on a cryostat and hybridization was performed as previously described.[51] RNA probes for mouse Islet-2 (a gift from Dr. S Pfaff), Chox-10 (a gift from Dr. R MacInnes), En1, Evx1 (gifts from Dr. M Goulding), Mash1 and Neurogenin-2 (gifts from Dr. F Guillmot) were prepared for hybridization.

Histological analysis and TUNEL staining

For histological analysis, embryos and yolk sacs were dissected at E10.5 and E11.5 and after whole-embryo culture, fixed in neutral formalin solution (pH 7.4), embedded in paraffin, and cut into 3.5 μm sections for hematoxylin and eosin staining. For *in situ* demonstration of apoptosis, TUNEL staining was performed according to the manufacturer's instructions (Wako, Japan). After labeling, samples were counter-stained with hematoxylin.

Immunohistochemical analysis

Frozen sections from E10.5 and E11.5 embryos were immunostained essentially as described[51] using anti-β-tubulin antibody (Sigma), anti-neurofilament antibody (2H3, Developmental Biology Hybridoma Bank), anti-Pax6 antibody[52] and anti-Islet-1 antibody (40.2D6, Developmental Biology Hybridoma Bank). Detection of immunoreactivity was performed using an ABC kit (Vector Laboratories).

Whole-mount immunohistochemistry

Whole-mount immunohistochemistry was performed as previously described.[53] Briefly, embryos dissected at E10.5 and yolk sacs prepared at E9.5, E10.5 and E11.5 were fixed in PBS containing 2% PFA for 2 h at 4°C, immersed in cold PBS overnight and treated with PBS containing 0.15% H_2O_2 and 0.1% NaN_3 at 4°C for 1 h. After washing with PBS, embryos and yolk sacs were preincubated in blocking solution (PBS–MBT) containing 2% milk, 0.2% bovine serum albumin, 0.6% goat serum and 0.1% Triton X-100 at 4°C for 1 h. Then, the samples were incubated with a rat monoclonal anti-PECAM-1 antibody (BD-PharMingen) in PBS–MBT at 4°C overnight and washed five times, 1 h each, with PBS–MT containing 2% milk and 0.1% Triton X-100 at room temperature. Subsequently, they were incubated with horseradish peroxidase-conjugated goat anti-rat IgG antibody (Cappel) in PBS–MBT at 4°C overnight, washed five times with PBS–MT, and developed with PBS containing 0.1% Triton X-100, 0.03% 3,3'-diaminobenzidine and 0.08% NiCl. After washing with PBS, embryos were destained with a mixture of benzyl alcohol and benzyl benzoate.

Acknowledgements

The authors would like to thank Drs. S Nagata, A Nagy and T Yagi for their gifts of JB-6 cells, RI cells and diphtheria toxin expression vector, Drs. R Kageyama, M Nozaki, K Koshimizu and H Yoshida for their valuable comments, and Ms. J Asami for excellent technical assistance. This work was supported in part by a Grant-in-Aid (12215070) from the Ministry of Education, Culture, Sports, Science and Technology of Japan to K Sakamaki.

References

1. Raff MC (1992) Social controls on cell survival and cell death. Nature 356: 397–400
2. Jacobson MD, Weil M and Raff MC (1997) Programmed cell death in animal development. Cell 88: 347–354
3. Vaux DL and Korsmeyer SJ (1999) Cell death in development. Cell 96: 245–254
4. Abbas AK (1996) Die and let live: eliminating dangerous lymphocytes. Cell 84: 655–657
5. Thornberry NA and Lazebnik Y (1998) Caspases: enemies within. Science 281: 1312–1316
6. Boldin MP, Goncharov TM, Goltsev YV and Wallach D (1996) Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. Cell 85: 803–815
7. Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J, Scaffidi C, Bretz JD, Zhang M, Gentz R, Mann M, Krammer PH, Peter ME and Dixit VM (1996) FLICE a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. Cell 85: 817–827
8. Ashkenazi A and Dixit VM (1998) Death receptors: signaling and modulation. Science 281: 1305–1308
9. Scaffidi C, Fulda S, Srinivasan A, Friesen C, Li F, Tomaselli KJ, Debatin KM, Krammer PH and Peter ME (1998) Two CD95 (APO-1/Fas) signaling pathways. EMBO J. 17: 1675–1687
10. Scaffidi C, Medema JP, Krammer PH and Peter ME (1997) FLICE is predominantly expressed as two functionally active isoforms, caspase-8/a and caspase-8/b. J. Biol. Chem. 272: 26953–26958
11. Luo X, Budihardjo I, Zou H, Slaughter C and Wang X (1998) Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell 94: 481–490
12. Varfolomeev EE, Schuchmann M, Luria V, Chiannilkulchai N, Beckmann JS, Mett IL, Rebrikov D, Brodianski VM, Kemper OC, Kollet O, Lapidot T, Soffer D, Sobe T, Avraham KB, Goncharov T, Holtmann H, Lonai P and Wallach D (1998) Targeted disruption of the mouse Caspase 8 gene ablates cell death induction by the TNF receptors, Fas/Apo1, and DR3 and is lethal prenatally. Immunity 9: 267–276
13. Bodmer JL, Holler N, Reynard S, Vinciguerra P, Schneider P, Juo P, Blenis J and Tschopp J (2000) TRAIL receptor-2 signals apoptosis through FADD and caspase-8. Nat. Cell Biol. 2: 241–243
14. Kischkel FC, Lawrence DA, Chuntharapai A, Schow P, Kim KJ and Ashkenazi A (2000) Apo2L/TRAIL-dependent recruitment of endogenous FADD and caspase-8 to death receptors 4 and 5. Immunity 12: 611–620
15. Sprick MR, Weigand MA, Rieser E, Rauch CT, Juo P, Blenis J, Krammer PH and Walczak H (2000) FADD/MORT1 and caspase-8 are recruited to TRAIL receptors 1 and 2 and are essential for apoptosis mediated by TRAIL receptor 2. Immunity 12: 599–609
16. Los M, Wesselborg S and Schulze-Osthoff K (1999) The role of caspases in development, immunity, and apoptotic signal transduction: lessons from knockout mice. Immunity 10: 629–639
17. Zheng TS and Flavell RA (2000) Divinations and surprises: genetic analysis of caspase function in mice. Exp. Cell Res. 256: 67–73
18. Kuida K, Zheng TS, Na S, Kuan C, Yang D, Karasuyama H, Rakic P and Flavell RA (1996) Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. Nature 384: 368–372
19. Kuida K, Haydar TF, Kuan CY, Gu Y, Taya C, Karasuyama H, Su MS, Rakic P and Flavell RA (1998) Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. Cell 94: 325–337
20. Hakem R, Hakem A, Duncan GS, Henderson JT, Woo M, Soengas MS, Elia A, de la Pompa JL, Kagi D, Khoo W, Potter J, Yoshida R, Kaufman SA, Lowe SW, Penninger JM and Mak TW (1998) Differential requirement for caspase 9 in apoptotic pathways *in vivo*. Cell 94: 339–352
21. Cecconi F, Alvarez-Bolado G, Meyer BI, Roth KA and Gruss P (1998) Apaf1 (CED-4 homolog) regulates programmed cell death in mammalian development. Cell 94: 727–737
22. Yoshida H, Kong YY, Yoshida R, Elia AJ, Hakem A, Hakem R, Penninger JM and Mak TW (1998) Apaf1 is required for mitochondrial pathways of apoptosis and brain development. Cell 94: 739–750
23. Yeh WC, Pompa JL, McCurrach ME, Shu HB, Elia AJ, Shahinian A, Ng M, Wakeham A, Khoo W, Mitchell K, El-Deiry WS, Lowe SW, Goeddel DV and Mak TW (1998) FADD: essential for embryo development and signaling from some, but not all, inducers of apoptosis. Science 279: 1954–1958
24. Zhang J, Cado D, Chen A, Kabra NH and Winoto A (1998) Fas-mediated apoptosis and activation-induced T-cell proliferation are defective in mice lacking FADD/Mort1. Nature 392: 296–300
25. Yeh WC, Itie A, Elia AJ, Ng M, Shu HB, Wakeham A, Mirtsos C, Suzuki N, Bonnard M, Goeddel DV and Mak TW (2000) Requirement for Casper (c-FLIP) in regulation of death receptor-induced apoptosis and embryonic development. Immunity 12: 633–642
26. Sakamaki K, Tsukumo S and Yonehara S (1998) Molecular cloning and characterization of mouse caspase-8. Eur. J. Biochem. 253: 399–405
27. Vincenz C and Dixit VM (1997) Fas-associated death domain protein interleukin-1beta-converting enzyme 2 (FLICE2), an ICE/Ced-3 homologue, is proximally involved in CD95- and p55-mediated death signaling. J. Biol. Chem. 272: 6578–6583
28. Osumi-Yamashita N, Ninomiya Y and Eto K (1997) Mammalian craniofacial embryology in vitro. Int. J. Dev. Biol. 41: 187–194
29. Risau W (1997) Mechanisms of angiogenesis. Nature 386: 671–674
30. Meeson AP, Argilla M, Ko K, Witte L and Lang RA (1999) VEGF deprivation-induced apoptosis is a component of programmed capillary regression. Development 126: 1407–1415
31. Artavanis-Tsakonas S, Rand MD and Lake RJ (1999) Notch signaling: cell fate control and signal integration in development. Science 284: 770–776
32. Krebs LT, Xue Y, Norton CR, Shutter JR, Maguire M, Sundberg JP, Gallahan D, Closson V, Kitajewski J, Callahan R, Smith GH, Stark KL and Gridley T (2000) Notch signaling is essential for vascular morphogenesis in mice. Genes Dev. 14: 1343–1352
33. Huppert SS, Le A, Schroeter EH, Mumm JS, Saxena MT, Milner LA and Kopan R (2000) Embryonic lethality in mice homozygous for a processing-deficient allele of Notch1. Nature 405: 966–970
34. Rones MS, McLaughlin KA, Raffin M and Mercola M (2000) Serrate and Notch specify cell fates in the heart field by suppressing cardiomyogenesis. Development 127: 3865–3876
35. French LE, Hahne M, Viard I, Radlgruber G, Zanone R, Becker K, Muller C and Tschopp J (1996) Fas and Fas ligand in embryos and adult mice: ligand expression in several immune-privileged tissues and coexpression in adult tissues characterized by apoptotic cell turnover. J. Cell Biol. 133: 335–343
36. Nishimura-Morita Y, Nose M, Inoue T and Yonehara S (1997) Amelioration of systemic autoimmune disease by the stimulation of apoptosis-promoting receptor Fas with anti-Fas mAb. Int. Immunol. 9: 1793–1799
37. Adachi M, Suematsu S, Kondo T, Ogasawara J, Tanaka T, Yoshida N and Nagata S (1995) Targeted mutation in the Fas gene causes hyperplasia in peripheral lymphoid organs and liver. Nat. Genet. 11: 294–300
38. Pfeffer K, Matsuyama T, Kundig TM, Wakeham A, Kishihara K, Shahinian A, Wiegmann K, Ohashi PS, Kronke M and Mak TW (1993) Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to L. monocytogenes infection. Cell 73: 457–467
39. Rothe J, Lesslauer W, Lotscher H, Lang Y, Koebel P, Kontgen F, Althage A, Zinkernagel R, Steinmetz M and Bluethmann H (1993) Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF- mediated toxicity but highly susceptible to infection by Listeria monocytogenes. Nature 364: 798–802
40. Wang EC, Thern A, Denzel A, Kitson A, Farrow SN and Owen MJ (2001) DR3 regulates negative selection during thymocyte development. Mol. Cell. Biol. 21: 3451–3461

41. Liu J, Na S, Glasebrook A, Fox N, Solenberg PJ, Zhang Q, Song HY and Yang DD (2001) Enhanced CD4+ T cell proliferation and Th2 cytokine production in DR6-deficeint mice. Immunity 15: 23–34
42. Cretney E, Takeda K, Yagita H, Glaccum M, Peschon JJ and Smyth MJ (2002) Increased susceptibility to tumor initiation and metastasis in TNF-related apoptosis-inducing ligand-deficient mice. J. Immunol. 168: 1356–1361
43. Yin XM, Wang K, Gross A, Zhao Y, Zinkel S, Klocke B, Roth KA and Korsmeyer SJ (1999) Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis. Nature 400: 886–891
44. Soriano P, Montgomery C, Geske R and Bradley A (1991) Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. Cell 64: 693–702
45. Yagi T, Nada S, Watanabe N, Tamemoto H, Kohmura N, Ikawa Y and Aizawa S (1993) A novel negative selection for homologous recombinants using diphtheria toxin A fragment gene. Anal. Biochem. 214: 77–86
46. Nagy A, Rossant J, Nagy R, Abramow-Newerly W and Roder J (1993) Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. Proc. Natl. Acad. Sci. USA 90: 8424–8428
47. Asano M, Furukawa K, Kido M, Matsumoto S, Umesaki Y, Kochibe N and Iwakura Y (1997) Growth retardation and early death of beta-1, 4-galactosyltransferase knockout mice with augmented proliferation and abnormal differentiation of epithelial cells. EMBO J. 16: 1850–1857
48. Sakamaki K, Tomonaga M, Tsukui K and Nagata S (1989) Molecular cloning and characterization of a chromosomal gene for human eosinophil peroxidase. J. Biol. Chem. 264: 16828–16836
49. Kawahara A, Ohsawa Y, Matsumura H, Uchiyama Y and Nagata S (1998) Caspase-independent cell killing by Fas-associated protein with death domain. J. Cell. Biol. 143: 1353–1360
50. Sakamaki K, Wang H-M, Miyajima I, Kitamura T, Todokoro K, Harada N and Miyajima A (1993) Ligand-dependent activation of chimeric receptors with the cytoplasmic domain of the interleukin-3 receptor β subunit (β_{IL3}). J. Biol. Chem. 268: 15833–15839
51. Osumi N, Hirota A, Ohuchi H, Nakafuku M, Iimura T, Kuratani S, Fujiwara M, Noji S and Eto K (1997) Pax-6 is involved in the specification of hindbrain motor neuron subtype. Development 124: 2961–2972
52. Inoue T, Nakamura S and Osumi N (2000) Fate mapping of the mouse prosencephalic neural plate. Dev. Biol. 219: 373–383
53. Yoshida H, Kunisada T, Kusakabe M, Nishikawa S and Nishikawa S-I (1996) Distinct stages of melanocyte differentiation revealed by analysis of nonuniform pigmentation patterns. Development 122: 1207–1214

ELSEVIER

Cytokine & Growth Factor Reviews 13 (2002) 341–355

Cytokine Growth Factor Reviews

www.elsevier.com/locate/cytogfr

Survey

Roles of IL-1 in the development of rheumatoid arthritis: consideration from mouse models

Yoichiro Iwakura*

Center for Experimental Medicine, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

Abstract

Expression of inflammatory cytokines is augmented in the joints of patients with rheumatoid arthritis (RA). We found that cytokine levels are also elevated in the joints of a mouse arthritis model, human T-cell leukemia virus type I (HTLV-I) transgenic (Tg) mouse. Depletion of IL-1 by gene targeting greatly reduced the incidence of the disease, indicating the importance of this cytokine in the development of arthritis. Furthermore, IL-1 receptor antagonist (IL-1Ra)-deficient mice develop autoimmunity and arthritis spontaneously. These observations suggest that excess IL-1 signaling the causes autoimmunity. We show that IL-1 activates the immune system non-specifically by inducing CD40L and OX40 co-signaling molecules on T cells. In this review, the roles of IL-1 in the development of autoimmunity and arthritis in mouse models will be discussed.

Keywords: Rheumatoid arthritis; IL-1; IL-1 receptor antagonist; OX 40; HTLV-I

Contents

1. Rheumatoid arthritis (RA)

RA is one of the most serious medical problems affecting approximately 1% of all people worldwide, irrespective of race. It is estimated that there are approximately 700,000 cases in Japan. The disease is autoimmune in nature and characterized by chronic inflammation of the synovial tissues in multiple joints that leads to joint destruction, but the etiopathogenesis has not been elucidated completely [1–3].

It is known that people who carry specific MHC haplotypes such as HLA-DR4 and -DR1 show a higher risk for development of chronic RA than those who carry other MHC molecules, suggesting the ability to present specific pathogenic antigens correlates to disease susceptibility [4–7]. On the other hand, infectious agents such as viruses, bacteria, and mycoplasmae have also been implicated in the development of arthritis based on findings that bacterial infections with mycobacteria and streptococci can induce arthritis in animals has been [8–10]. It shown that some bacterial components such as (heat shock protein: HSP65) or proteoglycans are immunologically cross-reactive with surface molecules on synovial cells [11]. Super antigens found in bacteria have also been implicated more generally in the development of autoimmunity [12].

The involvement of viral infections is also suggested by many studies. Infection with rubella virus has been shown to induce arthritis transiently [13], and EB virus [14] and

* Tel.: +81-3-5449-5536; fax: +81-5449-5430.
E-mail address: iwakura@ims.u-tokyo.ac.jp (Y. Iwakura).

1359-6101/02/$ – see front matter
PII: S1359-6101(02)00021-7

Table 1
Animal models for rheumatoid arthritis

(1) Spontaneous RA models
MRL-*lpr*/*lpr* mouse [25]
New Zealand Black mouse [27]
twy mouse [28]
SKG mouse [29]
(2) Induced RA models
Type II collagen-induced arthritis [30,32]
Adjuvant arthritis [34]
Streptococcal cell wall-induced arthritis [9]
Pristane-induced arthritis [161]
(3) Gene manipulated mouse RA models
HTLV-I transgenic mouse [20]
Human TNF-α transgenic mouse [38]
Human IL-1α transgenic mouse [40]
TNF-$\alpha^{\Delta ARE/\Delta ARE}$ mouse (3'AUUUA motif deletion) [39]
K/BxN mouse (a TCR transgenic mouse) [162]
BALB/c IL-1Ra-deficient mouse [41]

parvovirus [15] are also thought to have a similar effect; amino acid sequences of proteins from the EB virus show significant sequence homology with those of DR chains [16,17]. An association of arthritis with a retrovirus has also been reported [18]. Nishioka et al. first reported a patient with chronic inflammatory arthropathy infected with human T-cell leukemia virus type I (HTLV-I) [19], and Iwakura et al. found that HTLV-I could induce arthritis in mice [20]. Other retroviruses such as human immunodeficiency virus (HIV) [21] and caprine arthritis and encephalitis virus (CAEV) [22–24] are also known to induce arthritis.

Various animal disease models have been developed to elucidate the pathogenesis of the disease and to evaluate the efficacy of possible therapeutics (Table 1), including spontaneous, induced and gene-manipulated animal models. The MRL/*lpr* mouse, an established a model for systemic lupus erythematosus (SLE), and also develops arthritis spontaneously [25]. It is known that these mice carry a mutation in the *fas* gene, and T-cell apoptosis is impaired in these mice [26]. New Zealand Black [27] and twy [28] mice are also known to develop chronic arthritis. Recently, Sakaguchi reported the spontaneous development of arthritis in BALB/c mice (SKG mouse) [29]. These mice develop autoimmunity and the pathology of the joints closely resembles that of RA. The etiopathogenesis of these models, however, remains to be elucidated.

Collagen-induced arthritis (CIA) is a typical induced arthritis model, in which type II collagen, a major component is injected into rat, rabbit, mouse or monkey [30–32]. Injection of mycobacteruim cell wall complete Freund's adjuvant (CFA) [33,34], streptococcal cell wall [9], peptide glycans [10], or muramyl dipeptide [35] also induces acute joint inflammation. It has been shown that molecular mimicry occurs between cartilage antigens and a mycobacterium antigen HSP65 [36]. Streptococcal cell wall-induced arthritis is also protected by HSP65, suggesting that this heat shock protein is also a target for the T-cell response against streptococci [37]. These induced-RA models are especially useful for the analysis of effector phase mechanisms and evaluation of anti-inflammatory drugs.

Several RA models are produced using transgenic (Tg) techniques. Tg mice carrying a modified human TNF-α gene under its own promoter, which produces an ultra-stable TNF-α mRNA, develop arthritis characterized by subchondral evosions within 4 weeks of age [38]. Similar phenotypes were observed in mice lacking TNF AU-rich elements which mediate TNF-α mRNA destabilization [39]. Since this arthritis develops in an $RAG^{-/-}$ background, the involvement of an innate and/or stromal mechanism rather than an autoimmune mechanism has been suggested [39]. As will be described later, Tg mice carrying a human IL-1α gene [40] and IL-1 receptor antagonist (IL-1Ra)-deficient mice [41] also develop arthritis, indicating that IL-1, as well as TNF-α, plays an important role in the disease progression. Furthermore, we also developed a Tg arthritis model using the HTLV-I *tax* gene. Although each model has its own merits, the HTLV-I Tg mouse model is important, because epidemiological studies suggest that this virus actually causes RA in humans [42,43].

In spite of an enormous multidisciplinary effort, the etiopathogenesis of RA remains mostly unknown. The most widely accepted view is that autoimmunity underlies its development, and yet the mechanisms by which autoimmune reactions develop are just as poorly understood. Immunological cross-reactivity has been reported between some pathogenic antigens and native synovial components, while other arthritogenic agents, such as HTLV-I possess no cross-reactive agents. Thus, some other mechanisms may also be involved in the development of RA. In this review, I will discuss the pathogenic roles of cytokines, especially IL-1, in the development of arthritis and autoimmunity using HTLV-I Tg mouse and IL-1Ra-deficient mouse models.

2. HTLV-I Tg mouse model

HTLV-I is known as the causative agent of adult T-cell leukemia [44,45]. Approximately 1 million people are infected with this virus and 500–700 patients die from the leukemia every year in Japan. The virus encodes a transcriptional transactivator, Tax, in the *pX* region, that transactivates transcription from the cognate viral promoter through the 21 bp enhancer [46]. Encoding Tax also activates many cellular genes [44] including those for cytokines [47–50], cytokine receptors [51,52], and immediate early transcriptional factors [53,54] through activation of enhancers such as NF-κB-dependent enhancers or serum responsive elements [46].

Previously, we found that Tg mice carrying the HTLV-1 *env-pX* region with its own LTR promoter (HTLV-I Tg mice) developed chronic inflammatory polyarthropathy at a high incidence [20] (Table 2). The arthritis develops spontaneously in multiple joints as early as 4 weeks of age,

Table 2
Characteristics of HTLV-I transgenic mouse model

(1) Chronic, inflammatory polyarthropathy develops spontaneously at 4 weeks of age
(2) The *tax* gene is responsible for the development of arthritis
(3) The incidence differs among different mouse strain as follows: BALB/c, 60%; C3H/He, 20%; C57BL/6, 0% (at 3 months of age)
(4) Autoantibodies against IgG, type II collagen, and heat shock proteins are developed
(5) Development of arthritis is suppressed in athymic nude mice
(6) Arthritis can be induced by *tax*-transgenic bone marrow cell-transplantation, and suppressed by normal bone marrow cell-transplantation
(7) One of the autoantigens is type II collagen, and *tax*-transgenic mice are sensitive to immunization with type II collagen
(8) The *tax*-transgenic T cells are resistant against Fas-induced apoptosis, and over-expression of the Fas antigen ameliorates development of arthritis
(9) Expression of inflammatory cytokines including IL-1, IL-6, and TNF-α is augmented in the joints, and depletion of IL-1 or IL-6 reduces development of the disease

and at 3 months of age, 60% (BALB/cAn background) of the mice are affected. The histopathology is very similar to that of RA in humans, showing marked synovial and periarticular inflammation with articular erosion caused by invasion of granulation tissues [55]. These mice develop autoimmunity with elevated levels of antibodies against IgG, type II collagen, and HSPs [56], and show IgG hyper-gammaglobulinemia in which agalactosylated forms of the Ig carbohydrate chains increase [57].

We also produced Tg mice that expressed the *tax* gene alone under the control of either its own LTR or CD4 enhancer/promoter and found that both of these mice developed RA-like inflammatory arthropathy similar to HTLV-I Tg mice that carry the LTR-*env*-*pX*-LTR region, indicating that the *tax* gene itself is arthritogenic [58].

The incidence of arthritis differs greatly among different mouse strains [59]. When HTLV-I Tg mice were back-crossed with different inbred strains, the incidence of arthritis was as follows: 64 and 72% in BALB/cAn ($H\text{-}2^d$), 25 and 46% in C3H/HeN ($H\text{-}2^k$), and 0 and 2% in C57BL/6J ($H\text{-}2^b$) background at 3 and 6 months of age, respectively. Rheumatoid factor levels in the serum correlated with disease susceptibility, whereas IL-1β and MHC gene expression were similarly elevated in all these strains, suggesting in involvement of immune regulatory genes in the observed inter-strain difference. Introduction of the $H\text{-}2^d$ locus into C57BL/6J *pX*-Tg mice, however, did not increase disease incidence and substitution of the BALB/cAn *H-2* locus with $H\text{-}2^b$ did not decrease it. The results indicate that the *H-2* locus is not the major determinant of the disease, and that some other background genes specific to BALB/c mice contribute to the increased disease prevalence. Identification of these susceptibility genes may lead to the determination of their human homologs, many of which may be implicated in the multi-genetic development of human RA.

An autoimmune mechanism of pathogenesis was suggested in this model, because high levels of autoantibodies were detected in the serum, oligoclonal T cells were accumulated in the joints, and the disease onset was suppressed in athymic *nu/nu* mice [56,58–60]. Furthermore, when bone marrow (BM) and spleen cells from HTLV-I Tg mice were transferred into irradiated non-Tg mice, arthritis developed in these mice [61]. In contrast, arthritis in HTLV-I Tg mice was suppressed completely by the transplantation of non-Tg BM and spleen cells. Similar results were obtained by transferring BM cells only, indicating that the disease is not caused by the abnormal education of T cells in the periphery but by disorder in the stem cells.

T-cell receptor (TCR) β-chain variable region (Vβ) repertoires in the lymphatic organs are normal in Tg mice; however, specific Vβ positive T cells are expanded oligoclonally in the affected joints, suggesting that specific antigens, but not superantigens, were involved in the expansion of these T cells [60]. These expanded T cells had the same TCRs as those of lymph node T cells reactive to type II collagen. Moreover, these mice were susceptible to CIA and oligoclonal T cells of the same Vβ specificity as found in spontaneously-developed-arthritic joints, accumulated in the arthritic joints after immunization with type II collagen. These observations show that endogenous type II collagen is one of the arthritogenic antigens in the joint, suggesting a breakdown in tolerance to this antigen in HTLV-I Tg mice.

To elucidate the mechanisms of the development of autoimmunity, we examined the TCR Vβ repertoire [60]. We found that both TCR Vβ11- and Vβ12-positive populations were greatly reduced in the spleen and LNs in Tg mice and in non-Tg C3H/HeN mice by endogenous MMTV superantigens, indicating that negative selection in the thymus proceeds normally [60]. On the other hand, we found that T cells from HTLV-I Tg mice were refractory to anti-Fas treatment [62]. This finding suggests that this T cell defect may be involved in the development of autoimmunity in these mice, because Fas-mediated apoptosis of T cells is believed to be important in eliminating autoreactive T cells in the periphery [63]. In support of this idea, we showed that the incidence of autoimmunity increased when these HTLV-I Tg mice were crossed with *lpr/lpr* mice in which the *fas* gene is mutated, while it decreased when they were crossed with *fas*-Tg mice [59]. These observations suggest that a defect in Fas-mediated apoptosis of peripheral lymphocytes, rather than negative selection in the thymus is involved in the development of autoimmunity in HTLV-I Tg mice.

It seems difficult, however, to explain the development of autoimmunity solely by a defect in Fas-mediated apoptosis in HTLV-I Tg mice, because even *lpr/lpr* mice develop arthritis only in a specific genetic background and never de-

velop arthritis in the C3H/HeJ background, in which HTLV-I Tg mice develop arthritis. In this context, the oligocional expansion of Vβ12-positive T cells reactive with type II collagen in the affected joints [60] is interesting, because this finding suggests that T cells in the affected joints have recovered from the anergic state caused by MMTV superantigens. This tolerance break at the local sites could explain the development of autoimmunity in these HTLV-I Tg mice.

3. Suppression of arthritis development in IL-1-deficient mice

It is remarkable that various proinflammatory cytokine genes, including IL-1α, IL-1β, IL-2, IL-6, TNF-α and IFN-γ are activated in Tg joints [56], most likely due to the transcriptional transactivating activity of Tax (Fig. 1). Among them, IL-1 is especially interesting, because augmentation of IL-1 gene expression in the joints of RA patients has been reported by many investigators [64–70]. Production of IL-1 has also been reported in animal models such as antigen-induced arthritis in the rabbit and CIA in the mouse [71,72]. Furthermore, correlation of plasma IL-1 levels with disease activity exists in RA [73] and a genetic association between juvenile RA and an IL-1α polymorphism has been reported [74] (Fig. 2).

The arthritogenic activity of IL-1 is demonstrated by the following observations: (1) that injection of purified rIL-1 into rabbit knee joints induces the accumulation of polymorphonuclear and mononuclear leukocytes in the joint space and the loss of proteoglycan from the articular cartilage [75], (2) continuous infusion of human rIL-1α

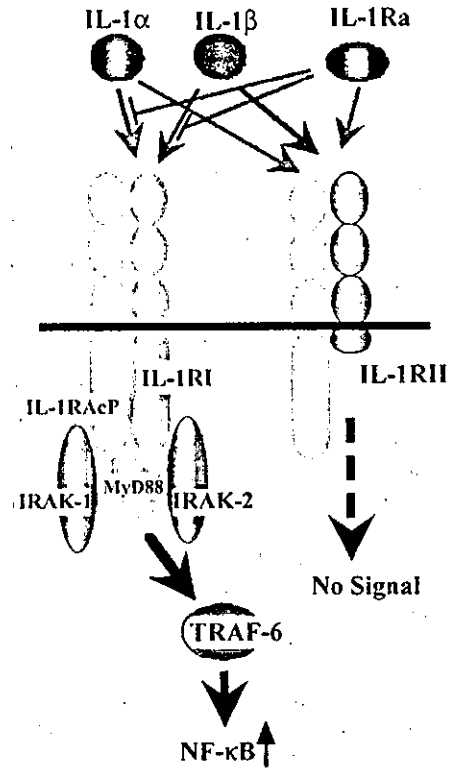

Fig. 2. The IL-1 system: the molecules and signal transduction.

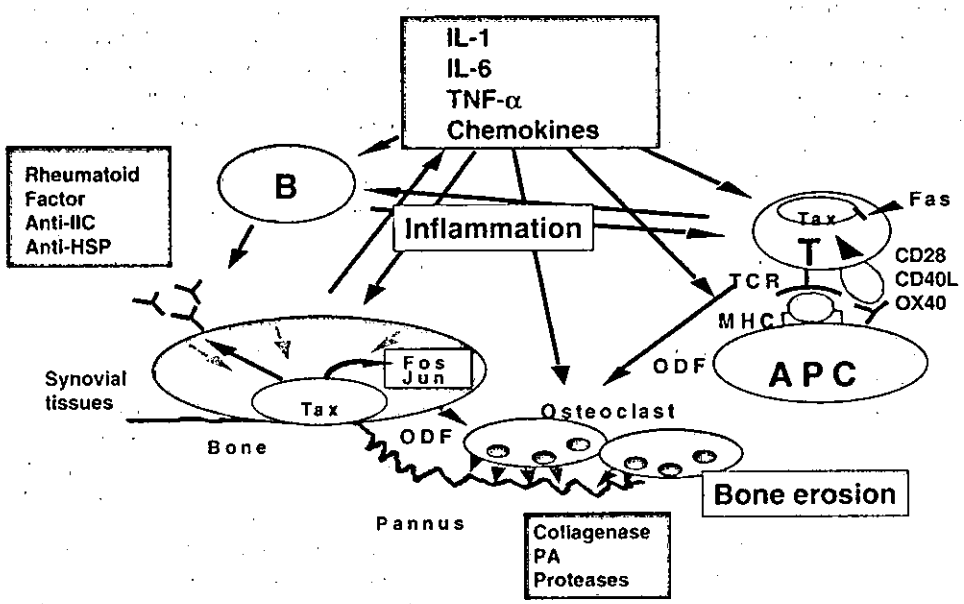

Fig. 1. Pathogenesis of arthritis caused by the HTLV-I *tax* gene.

into the rabbit knee joint induces arthritis [76], and (3) mouse rIL-1 induces an acute exacerbation of arthritis in rat joints previously injected with group A streptococcal peptidoglycan-polysaccharide [77]. These observations implicate IL-1 in the pathogenesis of RA.

We examined the effect of IL-1 deficiency on the development of arthritis in HTLV-I Tg mice. We found that the development of arthritis was suppressed markedly in IL-1-deficient mice. The incidence of arthritis was 60 and 80% at 3 and 6 months of age, respectively, in IL-$1^{+/+}$ HTLV-I Tg mice. In contrast, the corresponding rates in IL-$1\alpha/\beta^{-/-}$ mice are 10% and 30% at 3 and 6 months of age, respectively. The severity score of the affected mice was high, however, similar to IL-$1^{+/+}$ HTLV-I Tg mice, indicating that IL-1 plays an important role in the initial phase of the disease, but not as important in its later progression.

4. Suppression of CIA in IL-1-deficient mice

CIA is one of the most well-established RA models [30,78], and is induced in susceptible rodents by intradermal injections of homologous or heterologous native type II collagen. Susceptibility to the disease is dependent on MHC class II haplotypes; only mice with *H-2*^{q} and *H-2*^{r} haplotypes respond to immunization with type II collagen and develop arthritis [79]. Treatment of mice with either anti-TCR mAb or anti-CD4 mAb before immunization abrogates development of the disease and $CD4^+$ T-cell clones reactive to type II collagen transfer the disease to naive mice, indicating that MHC class II-restricted $CD4^+$ T-cell mediated immune reactions against type II collagen cause the disease [79]. Furthermore, since antibody levels against type II collagen correlated with the development of arthritis [79] and injection of antibodies against type II collagen induces arthritis [80], most investigators believe that both cellular and humoral immunity to type II collagen are necessary for the full development of CIA. Since antibodies against type II collagen are also detected in human cases, this model is considered to reproduce some aspects of the effector phase of RA [81,82].

We examined the effects of IL-1 deficiency using this RA model and found that the development of arthritis was severely suppressed in IL-1-deficient mice; 68% of wild-type mice developed arthritis 10 weeks after type II collagen immunization, compared to none (0%) of the IL-$1\alpha/\beta^{-/-}$ mice [83]. These results indicate that IL-1 also plays a crucial function in the development of CIA.

Disruption of either the IL-1α or IL-1β gene caused significant suppression of CIA. This was also true in the HTLV-I-Tg model; IL-1β-deficiency alone was enough to suppress the onset of the disease to the level observed in IL-$1\alpha/\beta^{-/-}$ mice. These results are unexpected because IL-1α and IL-1β should complement each other by acting on the same receptor [84,85]. The expression levels of IL-1α in IL-$1\beta^{-/-}$ mice and IL-1β in IL-$1\alpha^{-/-}$ mice after immunization with IIC were similar to those in wild-type mice. We conclude that IL-1α and IL-1β act synergistically, and deficiency in one of these molecules causes a great reduction in overall activity. Consistently with our results, it was reported that treatment with anti-IL-1α/β or anti-IL-β Ab ameliorated CIA in mice [86,87]. In these reports, however, anti-IL-1α antibody treatment showed no significant protection. It has also been reported that local expression of IL-1Ra protein can prevent murine CIA [88,89].

5. Roles of IL-1 in the development of arthritis

IL-1 is produced by various cell types, including macrophages, monocytes, and synovial lining cells, and induces inflammation by activating synovial cells, endothelial cells, lymphocytes and macrophages to produce various chemokines, cytokines, and inflammatory mediators [90]. These inflammatory mediators include IL-1 itself, IL-6, TNF-α, IL-8 and cyclooxygenase (COX)-2, and cause infiltration of inflammatory cells into inflammatory sites, permeability increases in blood vessels walls, and induction of fever [91–93]. Furthermore, IL-1 promotes synovial cell growth and activates synovial cells and osteoclasts to produce metalloproteases and collagenases that cause erosion of the bone and cartilage of joints [94]. Thus, it is possible that these activities of IL-1 are involved in the development of arthritis at the effector phase. Since the severity score of the arthritic mice in IL-1-deficient HTLV-I Tg mice, however, was not different from that of their wild-type counterparts, it was suggested that these IL-1 activities are not necessarily required to initiate the development of inflammation and not necessarily required in the effector phase [83].

In order to address the role of IL-1 in the development of arthritis, we analyzed the effects of IL-1 deficiency on the immune system. In the immune system, IL-1 is known to activate lymphocytes, monocytes, macrophages and NK cells [95,96]. When mice were immunized with protein antigens together with IL-1, serum antibody (Ab) production was enhanced, suggesting that IL-1 has an adjuvant effect [97,98].

Ab production after immunization with type II collagen in the presence of CFA in the CIA model was normal in IL-$1\alpha/\beta^{-/-}$ mice, while spontaneous autoantibody production against type II collagen was suppressed in IL-$1\alpha/\beta^{-/-}$ HTLV-I Tg mice [83]. Thus, IL-1 is required for the development of autoantibodies in HTLV-I Tg mice, but seems to not be required for the production of Abs against type II collagen in the CIA model. We reported previously that the presence of adjuvants abrogates the effect of IL-1 deficiency, because various cytokines such as TNF-α or IL-6, which have similar biological activities as IL-1, were induced by the treatment [99]. Nonetheless, CIA was severely suppressed in the IL-1-deficient mice. Thus, the function of IL-1 in enhancing Ab production seems to not be crucial for the development of arthritis. On the other hand, the T-cell proliferation response against type II collagen was reduced

greatly in both CIA and HTLV-I Tg models, indicating an inefficiency in T-cell priming in IL-$1^{-/-}$ mice [83]. Thus, it is suggested that IL-1 may play a crucial role in activating T cells that are required not only for the humoral, but also the cellular immune response. The role of IL-1 in the immune system was further analyzed using IL-$1Ra^{-/-}$ mice.

6. Development of autoimmune arthritis in IL-1Ra-deficient mice

IL-1Ra competes with IL-1α and IL-1β in binding IL-1 receptors but does not activate the receptor [100,101]. Three isoforms of IL-1Ra protein with inhibitory activity against IL-1 are synthesized by alternative splicing from a single gene; one is a secreted form with a signal polypeptide (sIL-1Ra), and the other two exist intracellularly (icIL-1RaI and icIL-1RaII) [102–105]. Thus, IL-1Ra is considered to be a negative regulator of IL-1 signals.

We have recently generated IL-1Ra-deficient mice and showed that these mice spontaneously developed chronic inflammatory arthropathy [41] (Table 3). The incidence of arthritis differs among different genetic backgrounds; the incidence is high in the BALB/c background, but low in the C57BL/6J background, similar to that observed in HTLV-I-induced arthritis, suggesting involvement of the same BALB/c-specific host genes. Arthritis starts to develop at 5 weeks of age, and almost all the mice were found to suffer from arthritis at 12 weeks of age in the BALB/c background mice. The histopathology of the lesion shows marked synovial and periarticular inflammation with articular erosion caused by invasion of granulation tissues, closely resembling RA in humans. Activation of osteoclasts is obvious at the pannus, and infiltration of inflammatory cells consisting mostly of neutrophils and fibrin clots are detected in the synovial space.

Total immunoglobulin levels as well as autoantibody levels against immunoglobulin, type II collagen and dsDNA are elevated in these mice [41]. When the *Rag2* gene in the IL-$1Ra^{-/-}$ mice was deleted, the development of arthritis was completely suppressed (Horai and Iwakura, unpublished observation). Furthermore, when T cells from IL-1Ra knockout mice were transferred to *nu/nu* mice, these mice developed arthritis (Horai and Iwakura, unpublished observation). These observations clearly indicate that excess IL-1 signal due to a deficiency in IL-1Ra causes autoimmunity, resulting in joint specific inflammation and bone destruction.

Recently, it was reported that Tg mice carrying the human IL-1α gene on the C3H/HeJ background developed arthritis spontaneously [40]. It was suggested that macrophages and polymorphonuclear neutrophils were directly involved in joint destruction; however, role of autoimmunity in the pathogenesis in this case was not evaluated.

Finally, we asked why IL-1Ra deficiency causes autoimmunity. We found that IL-1α, IL-1β and IL-1Ra were all constitutively expressed under physiological conditions in the joints of normal wild-type mice, and that the expression of IL-1β, but not IL-1α, increased two–three times in IL-1Ra knockout mice compared to wild-type controls [41]. The expression of other inflammatory cytokines such as TNF-α and IL-6 was also elevated, indicating a regulatory role IL-1Ra in the cytokine network. The elevation of cytokine expression in IL-1Ra knockout mice is considered to be caused by excess IL-1 signaling, because IL-1 can induce IL-6, TNF-α, and IL-1 itself [92,106]. Thus, in the absence of IL-1Ra, physiological levels of IL-1 induce pathogenic IL-1 responses resulting in the development of autoimmunity. These results were the first to suggest a link exists between excessive IL-1 signaling and autoimmunity, and that the balance between IL-1 and IL-1Ra is crucial for the homeostasis of the immune system.

7. T-cell sensitization with IL-1 through induction of CD40L and OX40

Since it was suggested that IL-1 plays a crucial role in activating the immune system, we then analyzed the mechanism through which it acts. First, we investigated the role of IL-1 in T-cell dependent (TD) Ab production using gene-targeted mice. Both primary and secondary Ab production against sheep red blood cells (SRBCs) were significantly reduced in IL-$1\alpha/\beta^{-/-}$ mice, and enhanced in IL-$1Ra^{-/-}$ mice [99]. The intrinsic functions of B cells such as Ab production against a type 1 T-independent antigen, trinitrophenyl (TNP)-LPS, and proliferative responses against mitogenic stimuli were normal in IL-$1\alpha/\beta^{-/-}$ mice. The proliferative response and cytokine production of T cells upon stimulation with anti-CD3 mAb were also normal, as was the phagocytotic ability of antigen-presenting

Table 3
Characteristics of IL-1 receptor antagonist-deficient mouse model

(1) Polyarthritis develops spontaneously at 5 weeks of age and almost all mice are affected by 13 weeks of age
(2) The incidence of arthritis differs among different strains of mice as follows: BALB/c, 80%; C57BL/6, 30% (at 3 months of age)
(3) Autoantibodies against IgG, type II collagen, and dsDNA are developed
(4) Arthritis can be induced by splenic T-cell transplantation or bone marrow cell transplantation, and suppressed by normal bone marrow cell transplantation
(5) Expression of inflammatory cytokines including IL-1, IL-6, and TNF-α are augmented in the joints, and TNF-α depletion suppresses development of the disease
(6) The development of arthritis can be suppressed significantly by the administration of anti-OX40L or anti-CD40L antibody

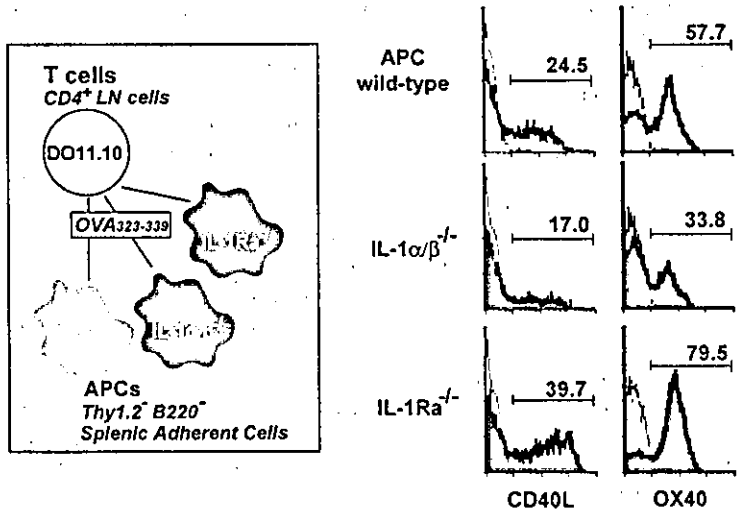

Fig. 3. Induction of CD40L and OX40 expression on T cells with IL-1.

cells (APCs). The proliferative response and cytokine production of T cells against SRBC, however, mediated by the interaction with APCs were markedly impaired in IL-1α/β$^{-/-}$ mice, and enhanced in IL-1Ra$^{-/-}$ mice. We found that SRBC-specific Ab production was reduced only in IL-1β$^{-/-}$ mice, but not in IL-1α$^{-/-}$ mice [107]. These results indicate that endogenous IL-1β, but not IL-1α, is involved in T-cell dependent Ab production, and IL-1 promotes antigen-specific T-cell helper function through T cell–APC interaction.

IL-1 function in the humoral immune response has also been examined using IL-1RI$^{-/-}$ mice [108,109]. These reports showed that specific serum Ab levels were normal in IL-1RI$^{-/-}$ mice when these mice were immunized with TNP-KLH/alum or TNP-KLH/CFA either intraperitoneally or subcutaneously. Furthermore, they showed that KLH-specific secondary T-cell proliferative responses were normal in these mice. Considered that adjuvant cause inflammation which consequently results in cytokine production at the site of injection, these apparent contradictions could be due to differences in immunization method. Since the functions of these inflammatory cytokines overlap partially, it is conceivable that the adjuvant effect of IL-1 could be complemented by some other cytokines such as TNF-α or IL-6.

Next, we examined the effects of IL-1 on T cell activation using an OVA-specific TCR Tg mouse, DO11.10. We found that IL-1$^{-/-}$ APCs did not fully activate DO11.10 T cells upon stimulation with OVA, while IL-1Ra$^{-/-}$ APCs were more efficient in T cell activation [99]. These observations indicate that IL-1 produced by APCs upon antigen binding promotes T-cell priming. Then, we analyzed molecules involved in the T cell–APC interaction, and found that the expression of cosignaling molecules, CD40 ligand (L) and OX40, on T cells was reduced upon interaction with IL-1$^{-/-}$ APCs and were increased upon interaction with IL-1Ra$^{-/-}$ APCs (Fig. 3). The expression of these molecules on T cells was induced directly with recombinant IL-1, although prior induction of IL-1R on T cells through TCR activation was required in naive T cells. Furthermore, the reduced Ab production in IL-1$^{-/-}$ mice was recovered by treatment with agonistic anti-CD40 mAb both in vitro and in vivo. These results indicate that IL-1 activates T cells by inducing CD40L and OX40 expression on T cells.

Similar enhancing effects of IL-1 were observed in contact hypersensitivity reaction [110]. We found that the proliferative response of TNP-specific T cells after sensitization with TNCB was reduced specifically in IL-1α$^{-/-}$ mice. Furthermore, adoptive transfer of TNP-conjugated IL-1-deficient epidermal cells into wild-type mice indicated that only IL-1α, but not IL-1β, produced by APCs in epidermal cells could prime allergen-specific T cells. A defect in T-cell priming was also observed in OVA-induced asthma in IL-1$^{-/-}$ mice (Nakae et al., unpublished observation).

8. Roles of the CD40L–CD40 and OX40L–OX40 system in the immune system

The CD40L (CD154) is a type II membrane glycoprotein that is expressed transiently on CD4$^+$ helper T cells after activation. Interactions between CD40L and CD40, its mitogenic receptor on B cells, provides the essential signal for the induction of B-cell activation and Ig production. Abnormal CD40L expression causes the Ig class switch defects observed in the X-linked hyper-IgM immunodeficiency syndrome, which is characterized by elevated levels of IgM and low levels of IgA, IgG, and IgE, the

absence of germinal centers, and the inability to mount TD humoral response, however, hyper-IgM syndrome is not observed in CD40L-deficient mice [111–113]. CD40 is also expressed by a large variety of cell types other than B cells, including dendritic cells, follicular dendritic cells, monocytes, macrophages, mast cells, fibroblasts, and endothelial cells [114]. Thus, the CD40–CD40L interaction is also important for the production of inflammatory cytokines, chemokines, and IL-12 from APCs, to induce cosignaling molecules, and to activate macrophages, NK cells, and endothelial cells [115]. Importantly, it has been shown that CD40 signaling can also induce another cosignaling molecule, OX40L, on APCs [116]. As a result, CD4 T-cell priming is enhanced through this interaction, and T-cell priming to both protein antigens and alloantigens is impaired in CD40L-deficient mice [117]. This interaction is also important for the enhancement of CD4 and CD8 T-cell clonal expansion, and protection from death of the activated T cells in the periphery [118].

On the other hand, a limited number of studies have suggested that priming of CD8 T cells is independent of CD40–CD40L, because infection of CD40L-deficient mice with lymphocytic choriomeningitis virus, Pichinde virus, or vesicular stomatitis virus can strongly activate primary CD8 cytotoxic T cells (CTLs) [119]. Antiviral CD8 CTL memory responses, however, were defective in CD40L-defective mice, suggesting a requirement of CD40L-mediated signaling in the establishment or maintenance of CTL memory [119,120]. Furthermore, involvement of CD40 signaling in the migration of antigen-specific dendritic cells from the skin to draining lymph nodes in contact hypersensitivity reaction [121], in the production of IFN-γ to evoke experimental allergic encephalomyelitis (EAE) [122], and in the development of atherosclerosis [123] were reported.

The role of CD40–CD40L signaling in the development of autoimmune reactions such as CIA, EAE, lupus nephritis, colitis, and oophoritis has been demonstrated in mouse models [124–127]. Treatment of mice with anti-CD40L antibodies blocks diseases development in these models, suggesting a role for the CD40–CD40L interaction either in the priming of T cells or in the effector functions that mediate tissue damage. Alternatively, anti-CD40L antibodies may block humoral responses that are believed to play a significant role in some autoimmune diseases such as arthritis and oophoritis. It has been reported that defective of CD40L–CD40 interactions are involved in neonatal transplantation tolerance [128], and excess CD40 signaling in the epidermis of CD40L Tg mice causes chronic skin inflammation and systemic autoimmunity [129].

OX40 (CD134) is another important cosignaling molecule that involves in the activation of T cells and APCs [130]. OX40 and OX40L is a member of the TNF receptor (R) and the TNF families, respectively, and is expressed on activated T cells and APCs [131,132], respectively. OX40 is not constitutively expressed, and is induced on naive T cells after stimulation with antigens or infection with HTLV-I [133]. OX40 is expressed most strongly on CD4 T-cell blasts in the T-cell zone [134]. OX40L is also inducible and CD40 signaling is important for inducing its expression on APCs [116]. OX40L is also expressed on HTLV-I-infected human leukemic T cells [131]. Several groups have shown that OX40L expressed on APCs can provide costimulation to CD4 T cells [135–139], and promotes T-cell survival through induction of Bcl-xL and Bcl-2 [140]. Activation of CD8 T cells has also been reported [141], although $OX40^{-/-}$ mice retain primary and memory CTL responses after infection with LCMV and influenza virus, in contrast to reduced CD4 T-cell proliferation and IFN-γ production [142].

OX40 stimulation of B cells increases immunoglobulin heavy chain mRNA levels and immunoglobulin secretion [143], and OX40–OX40L interaction is necessary for the differentiation of activated B cells into highly Ig-producing cells; however, it is not involved in other pathways of antigen-driven B-cell differentiation such as memory cell development in the germinal centers [144]. It was shown that T-cell priming was impaired in $OX40L^{-/-}$ mice accompanied by a concomitant reduction in both Th1 and Th2 cytokine production [116]. Antibody production against KLH and CTL induction was suppressed in those mice. Other investigators, however, reported that serum antigen-specific Ig levels were similar to wild-type mice when $OX40^{-/-}$ or $OX40L^{-/-}$ mice were immunized with various TD antigens including VSV, LCMV, TMEV, *Leishmania major*, *N. brasiliensis*, NP–CGG, TNP–KLH and TNP–OVA, indicating that the OX40–OX40L system is not required for Ab production under certain immunization conditions [142,145,146]. This apparent discrepancy suggests that differences in immunization conditions such as adjuvant, immunization route, or antigen dose may affect the results and that other signals can substitute for the defective OX40L.

$OX40^{-/-}$ mice exhibit an impaired contact hypersensitivity response due to defects in T-cell priming and cytokine production, suggesting that OX40L has a critical costimulatory function for dendritic cell–T cell interactions [145]. It is also reported that the OX40–OX40L system is involved in many diseases such as EAE [147,148], GVHD [149], colitis [132], and asthma [150]. Thus, OX40–OX40L interaction seems to not only regulate Ab production but also Th1-dependent cellular responses as well as CTL responses.

9. The role of IL-1 in the development of autoimmunity and arthritis

Collectively, the role of IL-1 in T cell and APC activation as understood to date, can be summed up as follows (Fig. 4). First, when APCs encounter an antigen, these cells produce IL-1 in response to the antigenic stimulation. On the other hand, IL-1R is induced on T cells via TCR signaling upon interaction with APCs. Then, APC-derived IL-1 activates T cells to induce CD40L and OX40, the former of which

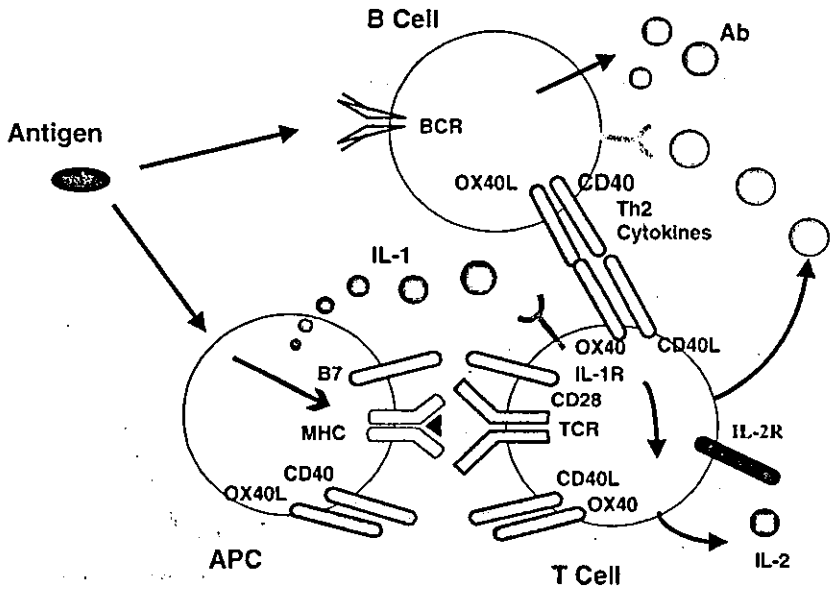

Fig. 4. Activation of the acquired immune system with IL-1.

activates APCs and B cells by interacting with constitutively expressed CD40 molecules on the surface of these cells leading induction of OX40L expression on their surfaces. This OX40L again activates T cells that are primed to express OX40 on the surface. Both CD4 T cells and CD8 T cells, which activate cellular and humoral immunity respectively, are activated by this interaction, resulting in the production of Th1 and Th2 cytokines, respectively.

Thus, over-expression of CD40L and OX40 can explain the development of autoimmunity in animals in which excess IL-1 signal is generated (Fig. 5). Accordingly, our findings that CD40L and OX40 expression on T cells are augmented in HTLV-I Tg mice and that this enhanced expression is normalized when IL-1 is deleted suggest that over-production of IL-1 causes autoimmunity through induction of CD40L and OX40 molecules on T cells [83]. Arthritis development in IL-1Ra$^{-/-}$ mice is also ameliorated when mice were treated with anti-OX40L or anti-CD40L Abs, suggesting that over-expression of these molecules is responsible for the autoimmune response (Horai et al., unpublished observation). Indeed, it has been reported recently that anti-OX40 Ab treatment is effective in treating CIA model mice [151]. Since CIA is induced, however, by enforced immunization against type II collagen, expression levels of OX40 are normal in these mice, in contrast to the augmented expression in HTLV-I Tg mice and IL-1Ra$^{-/-}$ mice. Thus, in this case, anti-OX40 Ab simply inhibits the T cell–APC interaction of the ordinary immune reaction.

Involvement of IL-1 in the development of autoimmunity is further supported by the following findings. We showed that C3H/HeN mice that are usually resistant against CIA became susceptible to treatment with type II collagen when the mice were introduced with HTLV-I *tax* gene, indicating that the immune system becomes hypersensitive against antigenic challenge in those mice [61]. Similar hyper-responsiveness was also observed in IL-1Ra$^{-/-}$ mice, suggesting that excess IL-1 signal is responsible for the hyper-responsiveness (Horai et al., unpublished observations). Consistently with this notion, it has been reported that exogenous IL-1 prevents tolerance induction by SEB [152], and that signals from an agonistic Ab against OX40 can break an existing state of tolerance in the CD4 T-cell compartment [153].

It is known that T cells become anergic when these cells encounter antigens without CD28 stimulation [154]. It has been shown, however, that Tg expression of the T-cell costimulator B7-1 (CD80) in the islets of Langerhans is not sufficient to abolish the in vivo tolerance to islet antigens, and coexpression of B7-1 and high levels of the class II MHC antigen I-E or TNF-α can induce autoimmune destruction of the b cells of the pancreas [155,156]. Our data indicate that IL-1 can activate T cells in the absence of CD28 stimulation, because only the signals from CD3 and IL-1R are sufficient to activate T cells [99]. It should be noted that the IL-1 and CD28 signaling pathways are independent, because CTLA4, a potent inhibitor of the CD28 pathway, could not inhibit IL-1-mediated T-cell activation [99]. Therefore, IL-1 can activate self-reactive T cells which are made anergic in the periphery due to lack of CD28 costimulation. Since TNF-α can induce IL-1 in vivo, the action of TNF-α in abolishing tolerance may actually be mediated by TNF-α-induced IL-1.

Thus, these findings indicate a crucial role for IL-1 in regulating the acquired immune system, augmenting its previously known role as a mediator of innate immunity. This

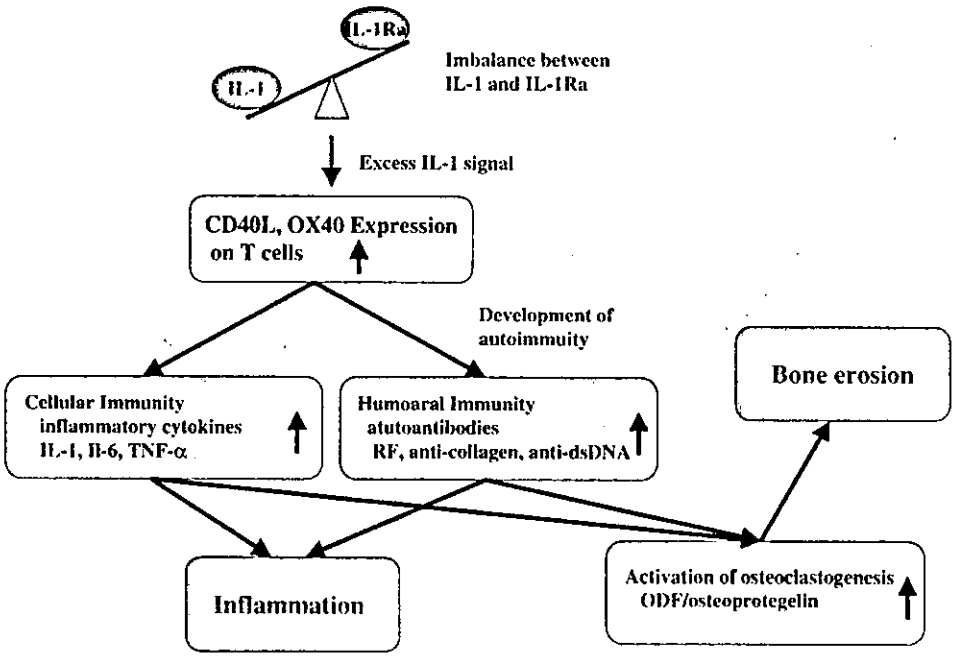

Fig. 5. Involvement of IL-1 in the development of autoimmunity and arthritis.

seems to be advantageous to the host defense mechanism, because the acquired immune system can be activated more efficiently by this mechanism upon infection. Under conditions in which excess IL-1 or excess IL-1 signal is produced, however, T cells become activated in excess, resulting in a breakdown of tolerance and development of autoimmunity.

It should be noted that cross-reactivity between pathogenic antigens and host autoantigens is not necessary for tolerance breakdown. In fact, no immunological cross-reactivity has been reported between HTLV-I and synovial tissues. The fact that IL-1Ra-deficiency causes autoimmunity clearly indicates that excess IL-1 signal is sufficient to cause an autoimmune response. Since self-components are protected from the immune system through several mechanisms [157], simple infection with pathogens immunologically cross-reactive with synovial tissues may not necessarily cause an autoimmune response, although molecular mimicry is widely believed to be a mechanism by which it can develop [158]. Most likely, cytokines are also involved in the development of autoimmunity in such cases. In support of this hypothesis, enhancing effect of CFA or inflammation on the development of autoimmunity upon immunization with cross-reactive molecules has been reported [159,160]. Other than microbial infections, either inflammation caused by mechanical injury or immune reaction, invasion of a transcriptional transactivator that can activate the IL-1 genes, or a genetic disorder in IL-1 or one of its regulatory genes, may also cause over production or excess signaling of this cytokine. Frequent development of arthritis after microbial infections such as mycobacteria or streptococci may be explained in part by this excess-IL-1-mediated mechanism.

Although IL-1 was first discovered as a major mediator of inflammation, it has gradually become evident that this cytokine has numerous functions related to host defense mechanisms, not only regulating the immune system, but also the areas of the neuronal and endocrine systems that interface with the immune system [92,93,95]. In fact it has been shown that IL-1 is induced in the brain upon inflammation and the associated development of fever was not observed in IL-$1^{-/-}$ mice and exaggerated in IL-$1Ra^{-/-}$ mice, indicating that IL-1 plays a crucial role in the development of fever by acting on the neuronal system [106]. Furthermore, we showed that IL-1 is also involved in the regulation of glucocorticoid secretion through the hypothalamus-pituitary–adrenal cortex axis [106]. Thus, IL-1 is considered to play critically important roles in the development of RA not only in inducing inflammatory responses but also activating the immune, the neuronal and the endocrine systems. The control of the production and activity of this cytokine should be critically important for the treatment of RA.

References

[1] Firestein GS, Zvaifler NJ. Rheumatoid arthritis: a disease of disordered immunity. In: Gallin JI, Goldstein IM, Snyderman R, editors. Inflammation: basic principles and clinical correlates. New York: Raven Press, 1992. p. 959–75.

[2] Cohen PL. Systemic autoimmunity. In: William EP, editor. Fundamental immunology. 4th ed. Philadelphia: Lippincott-Raven, 1999. p. 1067–88.
[3] Morrow J, Nelson JL, Watts R, Isenberg D. Rheumatoid arthritis. In: Autoimmune rheumatic diseases. 2nd ed. New York: Oxford University Press, 1999. p. 104–46.
[4] Stastny P. Association of the B-cell alloantigen DRw4 with rheumatoid arthritis. N Engl J Med 1978;298:869–71.
[5] Young A, Jaraquemada D, Awad J, Festenstein H, Corbett M, Hay FC, et al. Association of HLA-DR4/Dw4 and -DR2/Dw2 with radiologic changes in a prospective study of patients with rheumatoid arthritis: preferential relationship with HLA-Dw rather than HLA-DR specificities. Arthritis Rheum 1984;27:20–5.
[6] Duquesnoy RJ, Marrari M, Hackbarth S, Zeevi A. Serological and cellular definition of a new HLA-DR associated determinant, MC1, and its association with rheumatoid arthritis. Hum Immunol 1984;10:165–76.
[7] Goronzy J, Weyand CM, Fathman CG. Shared T-cell recognition sites on human histocompatibility leukocyte antigen class II molecules of patients with seropositive rheumatoid arthritis. J Clin Invest 1986;77:1042–9.
[8] Venables PJ. Infection and rheumatoid arthritis. Curr Opin Rheumatol 1989;1:15–30.
[9] DeJoy SQ, Ferguson KM, Sapp TM, Zabriskie JB, Oronsky AL, Kerwar SS. Streptococcal cell wall arthritis: passive transfer of disease with a T-cell line and cross-reactivity of streptococcal cell wall antigens with *Mycobacterium tuberculosis*. J Exp Med 1989;170:369–82.
[10] Koga T, Kakimoto K, Hirofuji T, Kotani S, Ohkuni H, Watanabe K, et al. Acute joint inflammation in mice after systemic injection of the cell wall, its peptidoglycan, and chemically defined peptidoglycan subunits from various bacteria. Infect Immun 1985;50:27–34.
[11] Kaufman SH. Heat shock proteins and the immune response. Immunol Today 1990;11:129–31.
[12] Paliard X, West SG, Lafferty JA, Clements JR, Kappler JW, Marrack P, et al. Evidence for the effects of a superantigen in rheumatoid arthritis. Science 1991;253:325–9.
[13] Smith CA, Petty RE, Tingle AJ. Rubella virus and arthritis. Rheum Dis Clin North Am 1987;13:265–74.
[14] Alspaugh MA, Henle G, Lennette ET, Henle W. Elevated levels of antibodies to Epstein-Barr virus antigens in sera and synovial fluids of patients with rheumatoid arthritis. J Clin Invest 1981;67:1134–40.
[15] Takahashi Y, Murai C, Shibata S, Munakata Y, Ishii T, Ishii K, et al. Human parvovirus B19 as a causative agent for rheumatoid arthritis. Proc Natl Acad Sci USA 1998;95:8227–32.
[16] Roudier J, Rhodes G, Petersen J, Vaughan JH, Carson DA. The Epstein-Barr virus glycoprotein gp110, a molecular link between HLA-DR4, HLA-DR1, and rheumatoid arthritis. Scand J Immunol 1988;27:367–71.
[17] Douvas A, Sobelman S. Multiple overlapping homologies between two rheumatoid antigens and immunosuppressive viruses. Proc Natl Acad Sci USA 1991;88:6328–32.
[18] Krieg AM, Steinberg AD. Retroviruses and autoimmunity. J Autoimmun 1990;3:137–66.
[19] Nishioka K, Maruyama I, Sato K, Kitajima I, Nakajima Y, Osame M. Chronic inflammatory arthropathy associated with HTLV-I. Lancet 1989;1:441.
[20] Iwakura Y, Tosu M, Yoshida E, Takiguchi M, Sato K, Kitajima I, et al. Induction of inflammatory arthropathy resembling rheumatoid arthritis in mice transgenic for HTLV-I. Science 1991;253:1026–8.
[21] Rynes RI, Goldenberg DL, DiGiacomo R, Olson R, Hussain M, Veazey J. Acquired immunodeficiency syndrome-associated arthritis. Am J Med 1988;84:810–6.
[22] Crawford TB, Adams DS, Cheevers WP, Cork LC. Chronic arthritis in goats caused by a retrovirus. Science 1980;207:997–9.
[23] Clements JE, Narayan O, Cork LC. Biochemical characterization of the virus causing leukoencephalitis and arthritis in goats. J Gen Virol 1980;50:423–7.
[24] Jutila MA, Banks KL. Increased macrophage division in the synovial fluid of goats infected with caprine arthritis-encephalitis virus. J Infect Dis 1988;157:1193–202.
[25] Hang L, Theofilopoulos AN, Dixon FJ. A spontaneous rheumatoid arthritis-like disease in MRL/l mice. J Exp Med 1982;155:1690–701.
[26] Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature 1992;356:314–7.
[27] Rordorf C, Schnebli HP, Baltz ML, Tennent GA, Pepys MB. The acute-phase response in (NZB × NZW)F1 and MRL/l mice. J Exp Med 1982;156:1268–73.
[28] Hosoya Y, Yoshimura Y, Higaki S. A new breed of mouse showing multiple osteochondral lesion: twy mouse. Ryumachi 1981;21:157.
[29] Sakaguchi S. Cause and etiological mechanism of rheumatic arthritis using animal models. Nihon Rinsho Meneki Gakkai Kaishi 2000;23:497–8.
[30] Trentham DE, Townes AS, Kang AH. Autoimmunity to type II collagen an experimental model of arthritis. J Exp Med 1977;146:857–68.
[31] Steffen C, Zeitlhofer J, Zielinski C, Endler AT, Menzel J. Acute autoimmune collagen-induced arthritis in rabbits. Z Rheumatol 1978;37:275–85.
[32] Courtenay JS, Dallman MJ, Dayan AD, Martin A, Mosedale B. Immunisation against heterologous type II collagen induces arthritis in mice. Nature 1980;283:666–8.
[33] Knight B, Katz DR, Isenberg DA, Ibrahim MA, Le Page S, Hutchings P, et al. Induction of adjuvant arthritis in mice. Clin Exp Immunol 1992;90:459–65.
[34] Pearson CM. Development of arthritis, periarthritis, and periostitis in rats given adjuvants. Proc Soc Exp Biol Med 1956;91:95–101.
[35] Koga T, Kakimoto K, Hirofuji T, Kotani S, Sumiyoshi A, Saisho K. Muramyl dipeptide induces acute joint inflammation in the mouse. Microbiol Immunol 1986;30:717–23.
[36] van Eden W, Holoshitz J, Nevo Z, Frenkel A, Klajman A, Cohen IR. Arthritis induced by a T-lymphocyte clone that responds to *Mycobacterium tuberculosis* and to cartilage proteoglycans. Proc Natl Acad Sci USA 1985;82:5117–20.
[37] van den Broek MF, Hogervorst EJ, Van Bruggen MC, van Eden W, van der Zee R, van den Berg WB. Protection against streptococcal cell wall-induced arthritis by pre-treatment with the 65 kDa mycobacterial heat shock protein. J Exp Med 1989;170:449–66.
[38] Keffer J, Probert L, Cazlaris H, Georgopoulos S, Kaslaris E, Kioussis D, et al. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. EMBO J 1991;10:4025–31.
[39] Kontoyiannis D, Pasparakis M, Pizarro TT, Cominelli F, Kollias G. Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies. Immunity 1999;10:387–98.
[40] Niki Y, Yamada H, Seki S, Kikuchi T, Takaishi H, Toyama Y, et al. Macrophage- and neutrophil-dominant arthritis in human IL-1α transgenic mice. J Clin Invest 2001;107:1127–35.
[41] Horai R, Saijo S, Tanioka H, Nakae S, Sudo K, Okahara A, et al. Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin-1 receptor antagonist-deficient mice. J Exp Med 2000;191:313–20 [in process citation].
[42] Nishioka K, Sumida T, Hasunuma T. Human T-lymphotropic virus type I in arthropathy and autoimmune disorders. Arthritis Rheum 1996;39:1410–8.
[43] Eguchi K, Origuchi T, Takashima H, Iwata K, Katamine S, Nagataki S. High seroprevalence of anti-HTLV-I antibody in rheumatoid arthritis. Arthritis Rheum 1996;39:463–6.
[44] Yoshida M. HTLV-1 *tax*: regulation of gene expression and disease. Trends Microbiol 1993;1:131–5.

[45] Sugamura K, Hinuma Y. Human retroviruses: HTLV-I and HTLV-II. In: Levy A, editor. The *Retroviridae*, vol. 2. New York: Plenum Press, 1993. p. 399–435.
[46] Yoshida M, Suzuki T, Fujusawa J, Hirai H. HTLV-I oncoprotein Tax and cellular transcription factors. Curr Top Microbiol Immunol 1995;193:131–48.
[47] Maruyama M, Shibuya H, Harada H, Hatakeyama M, Seiki M, Fujita T, et al. Evidence for aberrant activation of the interleukin-2 autocrine loop by HTLV-I-encoded p40x and T3/Ti complex triggering. Cell 1987;48:343–50.
[48] Siekevitz M, Feinberg MB, Holbrook N, Wong-Staal F, Greene WC. Activation of interleukin-2 and interleukin-2 receptor (*Tac*) promoter expression by the transactivator (*tat*) gene product of human T-cell leukemia virus, type I. Proc Natl Acad Sci USA 1987;84:5389–93.
[49] Kim S-J, Kehrl JH, Burton J, Tendler CL, Jeang K-T, Danielpour D, et al. Transactivation of the transforming growth factor 1 (TGF-β1) gene by human T-lymphotropic virus type I Tax: a potential mechanism for the increased production of TGF-β1 in adult T-cell leukemia. J Exp Med 1990;172:121–9.
[50] Miyatake S, Seiki M, Yoshida M, Arai K. T-cell activation signals and human T-cell leukemia virus type I-encoded p40x protein activate the mouse granulocyte–macrophage colony-stimulating factor gene through a common DNA element. Mol Cell Biol 1988;8:5581–7.
[51] Inoue J, Seiki M, Taniguchi T, Tsuru S, Yoshida M. Induction of interleukin-2 receptor gene expression by p40x-encoded by human T-cell leukemia virus type I. EMBO J 1986;5:2883–8.
[52] Green JE, Begley CG, Wagner DK, Waldmann TA, Jay G. Transactivation of granulocyte–macrophage colony-stimulating factor and the interleukin-2 receptor in transgenic mice carrying the human T-lymphotropic virus type 1 *tax* gene. Mol Cell Biol 1989;9:4731–7.
[53] Fujii M, Sassone-Corsi P, Verma IM. c-*fos* promoter transactivation by the Tax1 protein of human T-cell leukemia virus type I. Proc Natl Acad Sci USA 1988;85:8526–30.
[54] Fujii M, Niki T, Mori T, Matsuda T, Matsui M, Nomura N, et al. HTLV-I Tax induces expression of various immediate early serum responsive genes. Oncogene 1991;6:1023–9.
[55] Yamamoto H, Sekiguchi T, Itagaki K, Saijo S, Iwakura Y. Inflammatory polyarthritis in mice transgenic for human T-cell leukemia virus type I. Arthritis Rheum 1993;36:1612–20.
[56] Iwakura Y, Saijo S, Kioka Y, Nakayama-Yamada J, Itagaki K, Tosu M, et al. Autoimmunity induction by human T-cell leukemia virus type 1 in transgenic mice that develop chronic inflammatory arthropathy resembling rheumatoid arthritis in humans. J Immunol 1995;155:1588–98.
[57] Endo T, Iwakura Y, Kobata A. Structural changes in the N-linked sugar chains of serum immunoglobulin G of HTLV-I transgenic mice. Biochem Biophys Res Commun 1993;192:1004–10.
[58] Habu K, J Y, Asano M, Saijo S, Itagaki K, Horai R, et al. The HTLV-I *tax* gene is responsible for the development of both inflammatory polyarthropathy resembling rheumatoid arthritis and non-inflammatory ankylotic in transgenic mice. J Immunol 1999;162:2956–63.
[59] Iwakura Y, Itagaki K, Ishitsuka C, Yamasaki Y, Matsuzawa A, Yonehara S, et al. The development of autoimmune inflammatory arthropathy in mice transgenic for the human T-cell leukemia virus type 1 *env-pX* region is not dependent on *H-2* haplotypes and modified by the expression levels of Fas antigen. J Immunol 1998;161:6592–8.
[60] Kotani M, Tagawa Y, Iwakura Y. Involvement of autoimmunity against type II collagen in the development of arthritis in mice transgenic for the human T-cell leukemia virus type I *tax* gene. Eur J Immunol 1998;28:1–11.
[61] Saijo S, Kotani M, Habu K, Ishitsuka C, Yamamoto H, Sekiguchi T, et al. Bone marrow-derived cells are responsible for the development of autoimmune arthritis in human T-cell leukemia virus type I-transgenic mice and those of normal mice can suppress the disease. J Immunol 1999;163:5700–7.
[62] Kishi S, Saijo S, Arai M, Karasawa S, Ueda S, Kannagi M, et al. Resistance to Fas-mediated apoptosis of peripheral T cells in human T-lymphocyte virus type I (HTLV-I) transgenic mice with autoimmune arthropathy. J Exp Med 1997;186:57–64.
[63] Nagata S, Golstein P. The Fas death factor. Science 1995;267:1449–56.
[64] Nouri AM, Panayi GS, Goodman SM. Cytokines and the chronic inflammation of rheumatic disease. I. The presence of interleukin-1 in synovial fluids. Clin Exp Immunol 1984;55:295–302.
[65] Firestein GS, Alvaro-Gracia JM, Maki R, Alvaro-Garcia JM. Quantitative analysis of cytokine gene expression in rheumatoid arthritis. J Immunol 1990;144:3347–53.
[66] MacNaul KL, Hutchinson NI, Parsons JN, Bayne EK, Tocci MJ. Analysis of IL-1 and TNF-α gene expression in human rheumatoid synoviocytes and normal monocytes by in situ hybridization. J Immunol 1990;145:4154–66.
[67] Miyasaka N, Sato K, Goto M, Sasano M, Natsuyama M, Inoue K, et al. Augmented interleukin-1 production and HLA-DR expression in the synovium of rheumatoid arthritis patients: possible involvement in joint destruction. Arthritis Rheum 1988;31:480–6.
[68] Fontana A, Hengartner H, Weber E, Fehr K, Grob PJ, Cohen G. Interleukin-1 activity in the synovial fluid of patients with rheumatoid arthritis. Rheumatol Int 1982;2:49–53.
[69] Buchan G, Barrett K, Turner M, Chantry D, Maini RN, Feldmann M. Interleukin-1 and tumour necrosis factor mRNA expression in rheumatoid arthritis: prolonged production of IL-1α. Clin Exp Immunol 1988;73:449–55.
[70] Wood NC, Dickens E, Symons JA, Duff GW. In situ hybridization of interleukin-1 in CD14-positive cells in rheumatoid arthritis. Clin Immunol Immunopathol 1992;62:295–300.
[71] Marinova-Mutafchieva L, Williams RO, Mason LJ, Mauri C, Feldmann M, Maini RN. Dynamics of proinflammatory cytokine expression in the joints of mice with collagen-induced arthritis (CIA). Clin Exp Immunol 1997;107:507–12.
[72] Henderson B, Rowe FM, Bird CR, Gearing AJ. Production of interleukin-1 in the joint during the development of antigen-induced arthritis in the rabbit. Clin Exp Immunol 1988;74:371–6.
[73] Eastgate JA, Symons JA, Wood NC, Grinlinton FM, di Giovine FS, Duff GW. Correlation of plasma interleukin-1 levels with disease activity in rheumatoid arthritis. Lancet 1988;2:706–9.
[74] McDowell TL, Symons JA, Ploski R, Forre O, Duff GW. A genetic association between juvenile rheumatoid arthritis and a novel interleukin-1α polymorphism. Arthritis Rheum 1995;38:221–8.
[75] Pettipher ER, Higgs GA, Henderson B. Interleukin-1 induces leukocyte infiltration and cartilage proteoglycan degradation in the synovial joint. Proc Natl Acad Sci USA 1986;83:8749–53.
[76] Feige U, Karbowski A, Rordorf-Adam C, Pataki A. Arthritis induced by continuous infusion of hr-interleukin-1α into the rabbit knee-joint. Int J Tissue React 1989;11:225–38.
[77] Stimpson SA, Dalldorf FG, Otterness IG, Schwab JH. Exacerbation of arthritis by IL-1 in rat joints previously injured by peptidoglycan–polysaccharide. J Immunol 1988;140:2964–9.
[78] Holmdahl R, Andersson M, Goldschmidt TJ, Gustafsson K, Jansson L, Mo JA. Type II collagen autoimmunity in animals and provocations leading to arthritis. Immunol Rev 1990;118:193–232.
[79] Wooley PH, Luthra HS, Stuart JM, David CS. Type II collagen-induced arthritis in mice. I. Major histocompatibility complex (I region) linkage and antibody correlates. J Exp Med 1981;154:688–700.
[80] Stuart JM, Cremer MA, Townes AS, Kang AH. Type II collagen-induced arthritis in rats: passive transfer with serum and evidence that IgG anti-collagen antibodies can cause arthritis. J Exp Med 1982;155:1–16.
[81] Andriopoulos NA, Mestecky J, Miller EJ, Bradley EL. Antibodies to native and denatured collagens in sera of patients with rheumatoid arthritis. Arthritis Rheum 1976;19:613–7.

[82] Schwartz RS. Autoimmunity and autoimmune diseases. In: William EP, editor. Fundamental immunology. New York: Raven Press, 1993. p. 1033–97.
[83] Saijo S, Asano M, Horai R, Yamamoto H, Iwakura Y. Suppression of autoimmune arthritis in interleukin-1-deficient mice in which T-cell activation is impaired due to low levels of CD40 ligand and OX40 expression on T cells. Arthritis Rheum 2002;46:533–44.
[84] Sims JE, Gayle MA, Slack JL, Alderson MR, Bird TA, Giri JG, et al. Interleukin-1 signaling occurs exclusively via the type I receptor. Proc Natl Acad Sci USA 1993;90:6155–9.
[85] Sims JE, Dower SK. Interleukin-1 receptors. Eur Cytokine Netw 1994;5:539–46.
[86] van den Berg WB, Joosten LA, Helsen M, van de Loo FA. Amelioration of established murine collagen-induced arthritis with anti-IL-1 treatment. Clin Exp Immunol 1994;95:237–43.
[87] Joosten LA, Helsen MM, van de Loo FA, van den Berg WB. Anti-cytokine treatment of established type II collagen-induced arthritis in DBA/1 mice: a comparative study using anti-TNF-α, anti-IL-1α/β, and IL-1Ra. Arthritis Rheum 1996;39:797–809.
[88] Bakker AC, Joosten LA, Arntz OJ, Helsen MM, Bendele AM, van de Loo FA, et al. Prevention of murine collagen-induced arthritis in the knee and ipsilateral paw by local expression of human interleukin-1 receptor antagonist protein in the knee. Arthritis Rheum 1997;40:893–900.
[89] van de Loo FA, van den Berg WB. Gene therapy for rheumatoid arthritis: lessons from animal models, including studies on interleukin-4, interleukin-10, and interleukin-1 receptor antagonist as potential disease modulators. Rheum Dis Clin North Am 2002;28:127–49.
[90] Feldmann M, Brennan FM, Maini RN. Rheum. arthritis. Cell 1996;85:307–10.
[91] Davis P, MacIntyrek DE. Prostaglandins and inflammation. In: Galin JI, Goldstein IM, Snyderman R, editors. Inflammation: basic principles and clinical correlates. New York: Raven Press, 1992. p. 123–37.
[92] Dinarello CA. Biologic basis for interleukin-1 in disease. Blood 1996;87:2095–147.
[93] Tocci MJ, Schmidt JA. Interleukin-1: structure and function. In: Remick DG, Friedland JS, editors. Cytokines in health and disease. 2nd ed. New York: Marcel Dekker, 1997. p. 1–27.
[94] DeMarco DM, Zurier RB. Cytokines and rheumatology. In: Remick DG, Friedland JS, editors. Cytokines in health and disease. New York: Marcel Dekker, 1997. p. 463–85.
[95] Dinarello CA. Interleukin-1. Cytokine Growth Factor Rev 1997;8:253–65.
[96] Dinarello CA. Interleukin-1 and interleukin-1 antagonism. Blood 1991;77:1627–52.
[97] Reed SG, Pihl DL, Conlon PJ, Grabstein KH. IL-1 as adjuvant: role of T cells in the augmentation of specific antibody production by recombinant human IL-1α. J Immunol 1989;142:3129–33.
[98] Staruch MJ, Wood DD. The adjuvanticity of interleukin-1 in vivo. J Immunol 1983;130:2191–4.
[99] Nakae S, Asano M, Horai R, Sakaguchi N, Iwakura Y. IL-1 enhances T-cell dependent antibody production through induction of CD40 ligand and OX40 on T cells. J Immunol 2001;167:90–7.
[100] Carter DB, Deibel Jr MR, Dunn CJ, Tomich C-SC, Laborde AL, Slightom JL, et al. Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein. Nature 1990;344:633–8 [see comments].
[101] Hannum CH, Wilcox CJ, Arend WP, Joslin FG, Dripps DJ, Heimdal PL, et al. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. Nature 1990;343:336–40.
[102] Malyak M, Smith Jr MF, Abel AA, Hance KR, Arend WP. The differential production of three forms of IL-1 receptor antagonist by human neutrophils and monocytes. J Immunol 1998;161:2004–10.
[103] Haskill S, Martin G, Van Le L, Morris J, Peace A, Bigler CF, et al. cDNA cloning of an intracellular form of the human interleukin-1 receptor antagonist associated with epithelium. Proc Natl Acad Sci USA 1991;88:3681–5.
[104] Muzio M, Polentarutti N, Sironi M, Poli G, De Gioia L, Introna M, et al. Cloning and characterization of a new isoform of the interleukin-1 receptor antagonist. J Exp Med 1995;182:623–8.
[105] Malyak M, Guthridge JM, Hance KR, Dower SK, Freed JH, Arend WP. Characterization of a low molecular weight isoform of IL-1 receptor antagonist. J Immunol 1998;161:1997–2003.
[106] Horai R, Asano M, Sudo K, Kanuka H, Suzuki M, Nishihara M, et al. Production of mice deficient in genes for interleukin (IL)-1α, IL-1β, IL-1α/β, and IL-1 receptor antagonist shows that IL-1β is crucial in turpentine-induced fever development and glucocorticoid secretion. J Exp Med 1998;187:1463–75.
[107] Nakae S, Horai R, Asano M, Iwakura I. Interleukin-1β, but not interleukin-1α, is required for T-cell dependent antibody production. Immunology 2001;104:402–9.
[108] Satoskar AR, Okano M, Connaughton S, Raisanen-Sokolwski A, David JR, Labow M. Enhanced Th2-like responses in IL-1 type 1 receptor-deficient mice. Eur J Immunol 1998;28:2066–74.
[109] Glaccum MB, Stocking KL, Charrier K, Smith JL, Willis CR, Maliszewski C, et al. Phenotypic and functional characterization of mice that lack the type I receptor for IL-1. J Immunol 1997;159:3364–71.
[110] Nakae S, Naruse-Nakajima C, Sudo K, Horai R, Asano M, Iwakura Y. IL-1α, but not IL-1β, is required for contact-allergen-specific T-cell activation during the sensitization phase in contact hypersensitivity. Int Immunol 2001;13:1471–8.
[111] Korthauer U, Graf D, Mages HW, Briere F, Padayachee M, Malcolm S, et al. Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM. Nature 1993;361:539–41.
[112] DiSanto JP, Bonnefoy JY, Gauchat JF, Fischer A, de Saint Basile G. CD40 ligand mutations in X-linked immunodeficiency with hyper-IgM. Nature 1993;361:541–3.
[113] Ramesh N, Fuleihan R, Geha R. Molecular pathology of X-linked immunoglobulin deficiency with normal or elevated IgM (HIGMX-1). Immunol Rev 1994;138:87–104.
[114] Grewal IS, Flavell RA. CD40 and CD154 in cell-mediated immunity. Annu Rev Immunol 1998;16:111–35.
[115] Ferlin WG, von der Weid T, Cottrez F, Ferrick DA, Coffman RL, Howard MC. The induction of a protective response in *Leishmania major*-infected BALB/c mice with anti-CD40 mAb. Eur J Immunol 1998;28:525–31.
[116] Murata K, Ishii N, Takano H, Miura S, Ndhlovu LC, Nose M, et al. Impairment of antigen-presenting cell function in mice lacking expression of OX40 ligand. J Exp Med 2000;191:365–74.
[117] Grewal IS, Xu J, Flavell RA. Impairment of antigen-specific T-cell priming in mice lacking CD40 ligand. Nature 1995;378:617–20.
[118] Maxwell JR, Campbell JD, Kim CH, Vella AT. CD40 activation boosts T-cell immunity in vivo by enhancing T-cell clonal expansion and delaying peripheral T-cell deletion. J Immunol 1999;162:2024–34.
[119] Borrow P, Tishon A, Lee S, Xu J, Grewal IS, Oldstone MB, et al. CD40L-deficient mice show deficits in antiviral immunity and have an impaired memory $CD8^+$ CTL response. J Exp Med 1996;183:2129–42.
[120] Yang Y, Wilson JM. CD40-ligand-dependent T-cell activation: requirement of B7–CD28 signaling through CD40. Science 1996;273:1862–4.
[121] Moodycliffe AM, Shreedhar V, Ullrich SE, Walterscheid J, Bucana C, Kripke ML, et al. CD40–CD40 ligand interactions in vivo regulate migration of antigen-bearing dendritic cells from the skin to draining lymph nodes. J Exp Med 2000;191:2011–20.
[122] Grewal IS, Foellmer HG, Grewal KD, Xu J, Hardardottir F, Baron JL, et al. Requirement for CD40 ligand in costimulation induction, T-cell activation, and experimental allergic encephalomyelitis. Science 1996;273:1864–7.

[123] Schonbeck U, Sukhova GK, Shimizu K, Mach F, Libby P. Inhibition of CD40 signaling limits evolution of established atherosclerosis in mice. Proc Natl Acad Sci USA 2000;97:7458–63.
[124] Durie FH, Fava RA, Foy TM, Aruffo A, Ledbetter JA, Noelle RJ. Prevention of collagen-induced arthritis with an antibody to gp39, the ligand for CD40. Science 1993;261:1328–30.
[125] Mohan C, Shi Y, Laman JD, Datta SK. Interaction between CD40 and its ligand gp39 in the development of murine lupus nephritis. J Immunol 1995;154:1470–80.
[126] Gerritse K, Laman JD, Noelle RJ, Aruffo A, Ledbetter JA, Boersma WJ, et al. CD40–CD40 ligand interactions in experimental allergic encephalomyelitis and multiple sclerosis. Proc Natl Acad Sci USA 1996;93:2499–504.
[127] Griggs ND, Agersborg SS, Noelle RJ, Ledbetter JA, Linsley PS, Tung KS. The relative contribution of the CD28 and gp39 costimulatory pathways in the clonal expansion and pathogenic acquisition of self-reactive T cells. J Exp Med 1996;183:801–10.
[128] Flamand V, Donckier V, Demoor FX, Le Moine A, Matthys P, Vanderhaeghen ML, et al. CD40 ligation prevents neonatal induction of transplantation tolerance. J Immunol 1998;160:4666–9.
[129] Mehling A, Loser K, Varga G, Metze D, Luger TA, Schwarz T, et al. Overexpression of CD40 ligand in murine epidermis results in chronic skin inflammation and systemic autoimmunity. J Exp Med 2001;194:615–28.
[130] Mallett S, Fossum S, Barclay AN. Characterization of the MRC OX40 antigen of activated CD4 positive T lymphocytes—a molecule related to nerve growth factor receptor. EMBO J 1990;9:1063–8.
[131] Baum PR, Gayle III RB, Ramsdell F, Srinivasan S, Sorensen RA, Watson ML, et al. Molecular characterization of murine and human OX40/OX40 ligand systems: identification of a human OX40 ligand as the HTLV-1-regulated protein gp34. EMBO J 1994;13:3992–4001.
[132] Malmstrom V, Shipton D, Singh B, Al-Shamkhani A, Puklavec MJ, Barclay AN, et al. CD134L expression on dendritic cells in the mesenteric lymph nodes drives colitis in T-cell restored SCID mice. J Immunol 2001;166:6972–81.
[133] Higashimura N, Takasawa N, Tanaka Y, Nakamura M, Sugamura K. Induction of OX40, a receptor of gp34, on T cells by transacting transcriptional activator, Tax, of human T-cell leukemia virus type I. Jpn J Cancer Res 1996;87:227–31.
[134] Durkop H, Latza U, Himmelreich P, Stein H. Expression of the human OX40 (hOX40) antigen in normal and neoplastic tissues. Br J Haematol 1995;91:927–31.
[135] Gramaglia I, Weinberg AD, Lemon M, Croft M. OX40 ligand: a potent costimulatory molecule for sustaining primary CD4 T-cell responses. J Immunol 1998;161:6510–7.
[136] Akiba H, Oshima H, Takeda K, Atsuta M, Nakano H, Nakajima A, et al. CD28-independent costimulation of T cells by OX40 ligand and CD70 on activated B cells. J Immunol 1999;162:7058–66.
[137] Calderhead DM, Buhlmann JE, van den Eertwegh AJ, Claassen E, Noelle RJ, Fell HP. Cloning of mouse OX40: a T-cell activation marker that may mediate T–B-cell interactions. J Immunol 1993;151:5261–71.
[138] Godfrey WR, Fagnoni FF, Harara MA, Buck D, Engleman EG. Identification of a human OX40 ligand, a costimulator of $CD4^+$ T cells with homology to tumor necrosis factor. J Exp Med 1994;180:757–62.
[139] Gerloni M, Xiong S, Mukerjee S, Schoenberger SP, Croft M, Zanetti M. Functional cooperation between T-helper cell determinants. Proc Natl Acad Sci USA 2000;97:13269–74.
[140] Rogers PR, Song J, Gramaglia I, Killeen N, Croft M. OX40 promotes Bcl-xL and Bcl-2 expression and is essential for long-term survival of CD4 T cells. Immunity 2001;15:445–55.
[141] Wang HC, Klein JR. Multiple levels of activation of murine $CD8^+$ intraepithelial lymphocytes defined by OX40 (CD134) expression: effects on cell-mediated cytotoxicity, IFN-γ, and IL-10 regulation. J Immunol 2001;167:6717–23.
[142] Kopf M, Ruedl C, Schmitz N, Gallimore A, Lefrang K, Ecabert B, et al. OX40-deficient mice are defective in Th cell proliferation but are competent in generating B cell and CTL responses after virus infection. Immunity 1999;11:699–708.
[143] Stuber E, Neurath M, Calderhead D, Fell HP, Strober W. Cross-linking of OX40 ligand, a member of the TNF/NGF cytokine family, induces proliferation and differentiation in murine splenic B cells. Immunity 1995;2:507–21.
[144] Stuber E, Strober W. The T cell–B cell interaction via OX40–OX40L is necessary for the T-cell dependent humoral immune response. J Exp Med 1996;183:979–89.
[145] Chen AI, McAdam AJ, Buhlmann JE, Scott S, Lupher Jr ML, Greenfield EA, et al. OX40-ligand has a critical costimulatory role in dendritic cell: T-cell interactions. Immunity 1999;11:689–98.
[146] Pippig SD, Pena-Rossi C, Long J, Godfrey WR, Fowell DJ, Reiner SL, et al. Robust B-cell immunity but impaired T-cell proliferation in the absence of CD134 (OX40). J Immunol 1999;163:6520–9 [in process citation].
[147] Ndhlovu LC, Ishii N, Murata K, Sato T, Sugamura K. Critical involvement of OX40 ligand signals in the T-cell priming events during experimental autoimmune encephalomyelitis. J Immunol 2001;167:2991–9.
[148] Nohara C, Akiba H, Nakajima A, Inoue A, Koh CS, Ohshima H, et al. Amelioration of experimental autoimmune encephalomyelitis with anti-OX40 ligand monoclonal antibody: a critical role for OX40 ligand in migration, but not development, of pathogenic T cells. J Immunol 2001;166:2108–15.
[149] Kotani A, Ishikawa T, Matsumura Y, Ichinohe T, Ohno H, Hori T, et al. Correlation of peripheral blood $OX40^+(CD134^+)$ T cells with chronic graft-versus-host disease in patients who underwent allogeneic hematopoietic stem cell transplantation. Blood 2001;98:3162–4.
[150] Jember AG, Zuberi R, Liu FT, Croft M. Development of allergic inflammation in a murine model of asthma is dependent on the costimulatory receptor OX40. J Exp Med 2001;193:387–92.
[151] Yoshioka T, Nakajima A, Akiba H, Ishiwata T, Asano G, Yoshino S, et al. Contribution of OX40/OX40 ligand interaction to the pathogenesis of rheumatoid arthritis. Eur J Immunol 2000;30:2815–23.
[152] Nakata Y, Matsuda K, Uzawa A, Nomura M, Akashi M, Suzuki G. Administration of recombinant human IL-1 by *Staphylococcus* enterotoxin B prevents tolerance induction in vivo. J Immunol 1995;155:4231–5.
[153] Bansal-Pakala P, Jember AG, Croft M. Signaling through OX40 (CD134) breaks peripheral T-cell tolerance. Nat Med 2001;7:907–12.
[154] Ohashi PS, Oehen S, Buerki K, Pircher H, Ohashi CT, Odermatt B, et al. Ablation of tolerance and induction of diabetes by virus infection in viral antigen transgenic mice. Cell 1991;65:305–17.
[155] Guerder S, Meyerhoff J, Flavell R. The role of the T-cell costimulator B7-1 in autoimmunity and the induction and maintenance of tolerance to peripheral antigen. Immunity 1994;1:155–66.
[156] Guerder S, Picarella DE, Linsley PS, Flavell RA. Costimulator B7-1 confers antigen-presenting cell function to parenchymal tissue and in conjunction with tumor necrosis factor-α leads to autoimmunity in transgenic mice. Proc Natl Acad Sci USA 1994;91:5138–42.
[157] Shevach EM. Organ-specific autoimmunity. In: Fundamental immunology. 4th ed. Philadelphia: Lippincott-Raven, 1999. p. 1089–125.
[158] Schwartz RH. Immunological tolerance. In: Paul WE, editor. Fundamental immunology. 4th ed. Philadelphia: Lippincott-Raven, 1999. p. 701–39.
[159] Lehmann PV, Forsthuber T, Miller A, Sercarz EE. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. Nature 1992;358:155–7.

[160] Rose NR, Herskowitz A, Neumann DA, Neu N. Autoimmune myocarditis: a paradigm of post-infection autoimmune disease. Immunol Today 1988;9:117–20.
[161] Potter M, Wax JS. Genetics of susceptibility to pristane-induced plasmacytomas in BALB/cAn: reduced susceptibility in BALB/cJ with a brief description of pristane-induced arthritis. J Immunol 1981;127:1591–5.
[162] Kouskoff V, Korganow AS, Duchatelle V, Degott C, Benoist C, Mathis D. Organ-specific disease provoked by systemic autoimmunity. Cell 1996;87:811–22.

Immunity, Vol. 20, 25–35, January, 2004, Copyright ©2004 by Cell Press

RhoA and ζ PKC Control Distinct Modalities of LFA-1 Activation by Chemokines: Critical Role of LFA-1 Affinity Triggering in Lymphocyte In Vivo Homing

Cinzia Giagulli,[1] Elio Scarpini,[2] Linda Ottoboni,[1] Shuh Narumiya,[5] Eugene C. Butcher,[3,4] Gabriela Constantin,[1] and Carlo Laudanna[1,*]
[1]Department of Pathology
Faculty of Medicine
University of Verona
Strada Le Grazie 8
37134 Verona
Italy
[2]IRCCS Ospedale Maggiore
Department of Neurological Sciences
University of Milan
Via Sforza 35
20122 Milan
Italy
[3]Department of Pathology
Stanford University School of Medicine
Stanford, California 94305
[4]Center for Molecular Biology and Medicine
Veteran Affairs Health Care System
Palo Alto, California 94304
[5]Department of Pharmacology
Kyoto University Faculty of Medicine
Sakyo-ku, Kyoto 606-8501
Japan

Summary

Chemokines regulate rapid leukocyte adhesion by triggering a complex modality of integrin activation. We show that the small GTPase RhoA and the atypical ζ PKC differently control lymphocyte LFA-1 high-affinity state and rapid lateral mobility induced by chemokines. Activation of LFA-1 high-affinity state and lateral mobility is controlled by RhoA through the activity of distinct effector regions, demonstrating that RhoA is a central point of diversification of signaling pathways leading to both modalities of LFA-1 triggering. In contrast, ζ PKC controls LFA-1 lateral mobility but not affinity triggering. Blockade of the 23–40 RhoA effector region prevents induction of LFA-1 high-affinity state as well as lymphocyte arrest in Peyer's patch high endothelial venules. Thus, RhoA controls the induction of LFA-1 high-affinity state by chemokines independently of ζ PKC, and this is critical to support chemokine-regulated homing of circulating lymphocytes.

Introduction

The concerted action of adhesion molecules and chemokine receptors regulates leukocyte extravasation from the blood and determines the specificity of the immune response (Kunkel and Butcher, 2002). Chemokines activate an extremely rapid and complex modality of integrin activation consisting of heterodimer high-affinity state and of lateral mobility (Constantin et al., 2000; Laudanna et al., 2002). These modalities of integrin activation play a cooperative role in mediating LFA-1-dependent lymphocyte immediate arrest on ICAM-1 under physiologic flow conditions. In particular, induction of rapid LFA-1 lateral mobility on the plasma membrane has been shown to mediate lymphocyte arrest to surfaces presenting a low density of ICAM-1 (Constantin et al., 2000). This suggests that LFA-1 lateral mobility allows the adaptation of lymphocytes to blood vessels presenting a variable expression level of integrin ligand. In contrast, the physiological role of LFA-1 high-affinity state induction in the context of lymphocyte rapid adhesion and in vivo arrest has never been formally demonstrated.

Signaling pathways controlling LFA-1 activation are largely unknown. Recent data show the involvement of phosphatidylinositol 3(-OH) kinase (PI(3)K), Cytohesin-1, and Rap1 in LFA-1 lateral mobility induced by chemokines in lymphocytes (Constantin et al., 2000; Weber et al., 2001a; Shimonaka et al., 2003). However, signaling events controlling the rapid induction of LFA-1 high-affinity state by chemokines are completely unidentified.

Previous data have implicated the small GTPase RhoA and the atypical ζ PKC in chemoattractant-induced β1 and β2 integrin-mediated leukocyte adhesion (Laudanna et al., 1996, 1998). The discovery that chemokines activate in lymphocytes a complex modality of integrin activation raises the hypothesis that RhoA and ζ PKC may control specific modalities of LFA-1 triggering.

In this study we addressed the possibility that RhoA and ζ PKC may control different modalities of LFA-1 activation by chemokines. We found that RhoA and ζ PKC play diversified, yet necessary, roles in rapid LFA-1 triggering by chemokines in lymphocytes. RhoA controls both LFA-1 high-affinity state and lateral mobility induction by chemokines through the engagement of distinct RhoA downstream effector regions. In contrast, ζ PKC is involved only in heterodimer lateral mobility induction. Importantly, we show that blockade of RhoA downstream effector region specifically involved in the induction of LFA-1 high-affinity state prevents rapid arrest of circulating naive lymphocytes on ICAM-1-expressing high endothelial venules (HEV) in secondary lymphoid organs. These findings show that RhoA and ζ PKC are critical points of diversification in signal transduction pathways generated by chemokines and leading to LFA-1 activation. Moreover, the data illuminate the critical physiological role of triggering LFA-1 to high-affinity state in lymphocyte homing in vivo.

Results

A Novel Method for Analyzing RhoA Signaling Activity

Three distinct regions of RhoA have been shown to activate individual downstream effectors (Fujisawa et al., 1998). To block RhoA-dependent signaling in a region-selective manner, we generated fusion peptides

*Correspondence: carlo.laudanna@univr.it

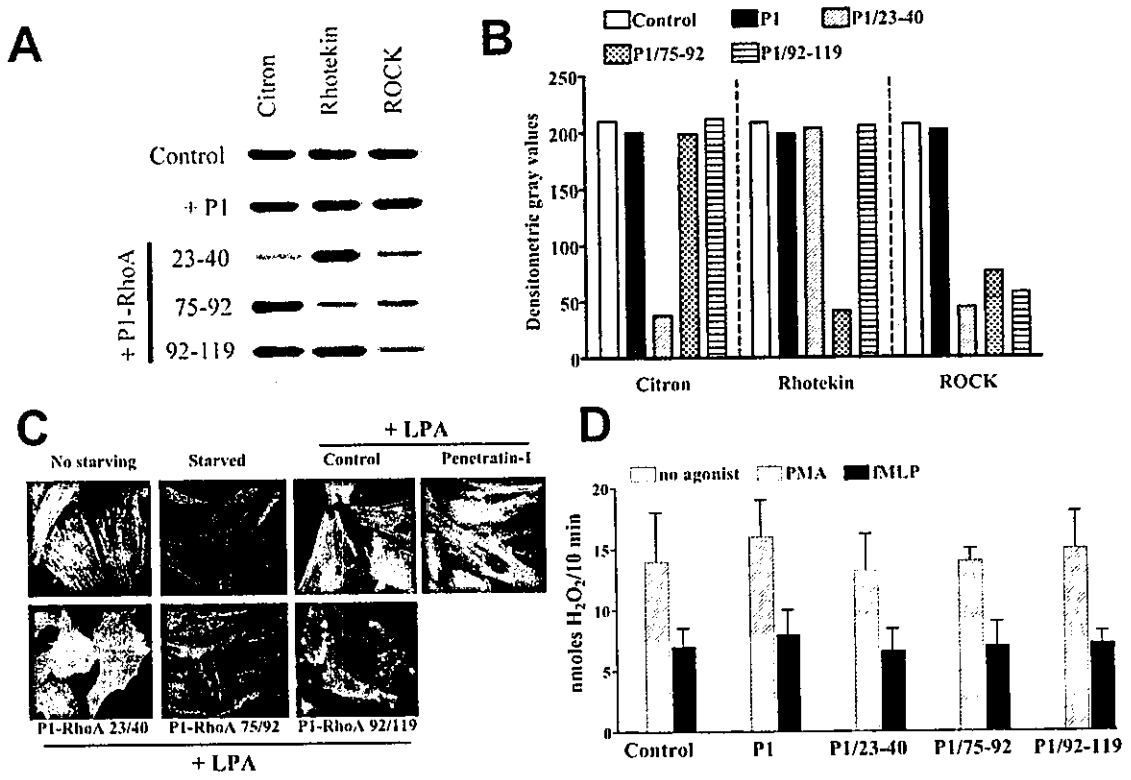

Figure 1. P1-RhoA Peptides Are Effective Inhibitors of RhoA-Dependent Signaling

(A) RhoA binding to Citron-, Rhotekin- and ROCK-RBD in the presence of buffer (Control) or 20 μg of Penetratin-1 (P1) or 23–40, 75–92, and 92–119 P1-RhoA peptides. A protein immunoblot of anti-RhoA is shown. One representative experiment of two.

(B) Densitometric analysis of the immunoblot shown in (A).

(C) Swiss 3T3 mouse fibroblasts were treated for 4 hr at 37°C with 50 μM of Penetratin-1 (P1) or different P1-RhoA peptides and then stimulated for 10 min with 25 ng/ml lysophosphatidic acid (LPA). Shown are confocal microscopy images.

(D) Human polymorphonuclear neutrophils were treated for 2 hr at 37°C with 50 μM of P1 or different P1-RhoA peptides and then stimulated with 10 ng/ml PMA or 100 nM formyl-Met-Leu-Phe (fMLP). Stimulation was performed under stirring at 37°C. Shown are nmoles of released H_2O_2. Values are the means from three experiments. Error bars are SDs.

including an N-terminal plasma membrane translocating sequence from the third helix of the homeoregion of *Drosophila melanogaster* transcription factor Antennapedia, also called Penetratin-1 (P1) (Prochiantz, 2000), fused to the three distinct downstream effector regions of human RhoA, encompassing amino acids 23–40, 75–92, and 92–119.

We first evaluated the ability of the peptides to block RhoA interaction with specific effectors. In pull-down assays, binding of RhoA to Citron was blocked by 23–40, but not 75–92 and 92–119, peptides; binding to Rhotekin was blocked by 75–92, but not 23–40 and 92–119, peptides; finally, binding to ROCK was inhibited by all the peptides (Figures 1A and 1B). These data are in agreement with a previous study (Fujisawa et al., 1998) and show that soluble RhoA-derived effector regions may block RhoA interaction with specific effectors. As the three peptides were able to significantly prevent RhoA interaction with ROCK, we could validate the biological activity of the peptides by testing the capability of P1-RhoA peptides to interfere with the accumulation of actin stress fibers induced by lysophosphatidic acid (LPA) in fibroblasts, a phenomenon dependent on RhoA-activated Rho-kinase (ROCK) (Amano et al., 1997). Pretreatment of fibroblasts with each peptide almost completely prevented the accumulation of stress fibers upon LPA triggering. In contrast, the peptide encompassing only the P1 sequence was completely ineffective (Figure 1C). Inhibition was dose dependent with significant effects starting at 10 μM (data not shown). These results are consistent with the known involvement of these RhoA sites in ROCK activation and with the result of our pull-down experiment. In contrast, LPA-induced membrane ruffling, which is Rac1 dependent (Ridley and Hall, 1992), was unaffected by pretreatment with the peptides (data not shown). Furthermore, in human polymorphonuclear neutrophils, the peptides were unable to block the phorbol myristate acetate (PMA)- and formyl-Met-Leu-Phe (fMLP)-induced activation of the superoxide forming complex NADPH-oxidase, whose activation relies on rac activity (Abo et al., 1991) (Figure 1D). Together, these data show that P1-RhoA fusion effector regions are effective selective inhibitors of RhoA-induced signaling events.