Psoriasis-Like Cutaneous Inflammation in Mice Lacking Interleukin-1 Receptor Antagonist

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Interleukin-1 receptor antagonist-deficient (*Il1rn*−/−) BALB/c mice developed inflammation localized to the skin of the ear pinna in 64% of the cases examined. Histopathologically, the disease had many features resembling human psoriasis, suggesting that it might be a useful disease model. The epidermis became thickened and hypertrophic, and expressed the immature keratin, K6, throughout. The stratum corneum showed parakeratotsis. Large epidermal projections formed into a grossly thickened dermis and both tissues were infiltrated by leukocytes. Neutrophil-rich microabscesses formed beneath the stratum corneum. Dendritic cells and activated T cells of both helper classes were identified in both the dermis and epidermis, while a high density of macrophages was seen in the dermis, where mast cells were also prominent. Dense patterns of apparently activated small dermal vessels were seen in the diseased dermis. Cutaneous inflammation, along with arterial inflammation and arthritis, is the third site-specific, inflammatory disease to be found to affect *Il1rn*−/− BALB/c mice. None of the diseases affected *Il1rn*−/− C57BL/6. In F2 hybrids of *Il1rn*−/− BALB/c and C57BL/6, cutaneous inflammation was absent, aortic inflammation was common, and arthritis was rare, indicating that the sets of background modifier genes that cause susceptibility to each disease are not fully overlapping.

Key words: chronic disease/mice, knockout/inflammation/interleukin 1
J Invest Dermatol 122:665–669, 2004

IL-1 receptor antagonist (IL-1ra) is a homolog and antagonist of the proinflammatory cytokines IL-1 and IL-1β (reviewed by Dinarello, 1996), inhibiting their binding to the functional type I IL-1 receptor (IL-1R1). Two molecular forms of IL-1ra have been described in the mouse, and are encoded by a single gene (*Il1rn*) (Gabay *et al*, 1997). We and others have previously shown that mice that are deficient in all forms of IL-1ra slowly develop large vessel arterial inflammation and rheumatoid-like arthritis, which are of moderate to high penetrance, but are strain dependent (Horai *et al*, 2000; Nicklin *et al*, 2000). The appearance of the two diseases is consistent with their being immune response mediated, and possibly autoimmune, since the inflammatory lesions in both cases have abundant antigen-presenting cells and $CD4^+$ cells that contain IFN-γ. Recently it has been reported that the arthritis is T cell dependent and may be transferred to *nu/nu* mice by T cells (Iwakura, 2002) from affected mice. The cutaneous inflammation that we describe here is therefore the third type of inflammatory lesion that has been observed at high penetrance in *Il1rn*−/− mice.

Abbreviations: DC, dendritic cell; IL, interleukin; IL-1ra, interleukin-1 receptor antagonist; *Il1rn*, mouse IL-1ra gene; MΦ, macrophage

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Results and Discussion

Psoriasis-like lesions in the ear pinnae of BALB/c *Il1rn*−/− mice Human psoriasis is characterized by the formation of scaly reddened plaques in susceptible areas of the skin. Both the epidermis and the dermis are involved. Immature keratinocytes go through accelerated, disorganized cycles of proliferation, the epidermis becomes thickened, and the rete ridges become elongated, projecting deeply into the dermis. The dermis becomes thickened and heavily infiltrated by leukocytes. *Il1rn*−/− BALB/c (Harlan) frequently developed inflamed and thickened patches on their ear pinnae. The disease was generally symmetrical and not influenced by the site of clipping for identification. Other areas of the body were seldom involved. Figure 1*a* shows the typical appearance of the early disease. To determine disease status, hematoxylin and eosin-stained sections (either formalin fixed or frozen) of mouse ear pinnae were assessed by two observers. If the epidermis was convoluted to form pegs, the tissue and animal were classed as affected. For illustration, normal skin from a wild type (Fig 1*b*) is compared with unaffected (Fig 1*c*), affected (Fig 1*d*), and the end-stage affected (Fig 1*e–g*) pathology from *Il1rn*−/−. Like human psoriasis, nuclei seemed to be retained in the outer layer of the stratum corneum (Fig 1*k*) (indicated by small arrows). Microabscess formation, as seen in human psoriasis, is seen in severely affected skin (Fig 1*e–g*, marked A). In Fig 1*h*, neutrophils (magenta) are stained specifically. In Fig 1*i*, mast cells

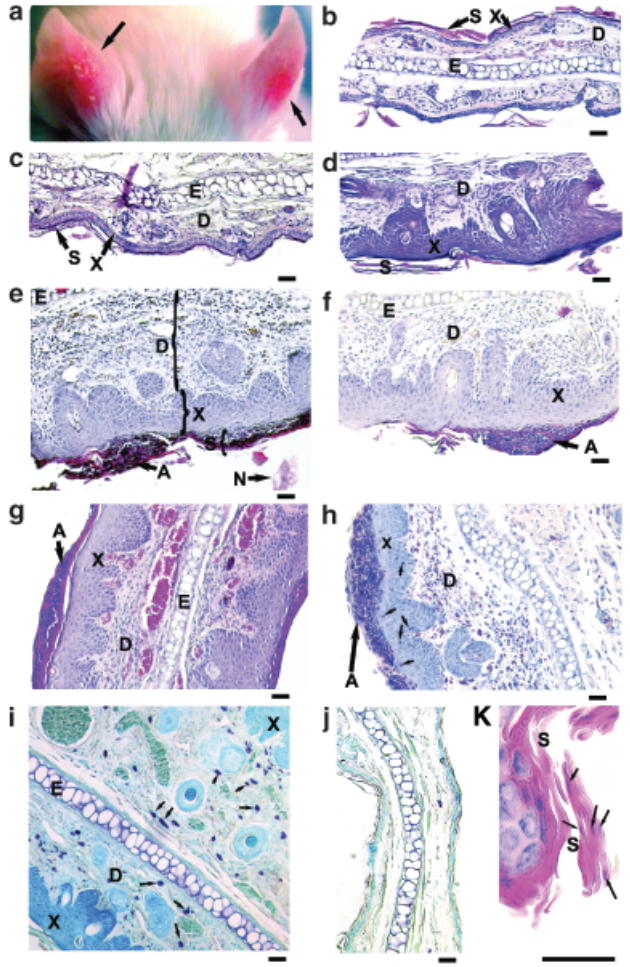

Figure 1
Histology of cutaneous inflammation. (*a*) Localized lesions (arrows) in a living 155 d *Il1rn*−/− BALB/c male. (*b–k*): Formalin-fixed transverse sections of ear skin from *Il1rn*+/+ and *Il1rn*−/− BALB/c. Labels: A, microabscess; D, dermis; E, elastic cartilage; S, stratum corneum; X, subcorneal epidermis. Scale bars are 50 μm. (*B–G*) were stained with hematoxylin and eosin. (*b*) Wild-type 224 d female. (*c*) Unaffected *Il1rn*−/− male. (*d*) Affected 154 d *Il1rn*−/− male without microabscesses. (*e–h*) Affected *Il1rn*−/− mice with microabscesses. (*e*) 83 d female (note N, which appears to be a sloughed nucleated squame). (*f*) 130 d female. (*g*) 199 d female. (*h*) Well-formed microabscess from the same mouse as (e), stained for neutrophil/mast cell-specific chloroacetate esterase (magenta) and counterstained with hematoxylin. Arrows: single neutrophils in the epidermis. (*i*, *j*) Toluidine blue staining. Arrows: mast cells. (*i*) 174 d *Il1rn–/–* male. (*j*) 155 d wild-type female. (*k*) Stratum corneum of a severely affected 130 d female, as in (*f*), showing apparently nucleated squames (arrows).

(indicated by small arrows) were found to be frequent in the affected dermis, compared with unaffected wild-type tissue (Fig 1*j*), a feature shared with human psoriasis.

Overall, we examined fresh-frozen and archived formaldehyde-fixed specimens of ear pinnae from 33 *Il1rn*−/− BALB/c from 18 litters, eight *Il1rn*+/−, and seven wild-type from the same litters. *Il1rn*−/− mice were euthanized between 71 and 316 d for a variety of reasons, including malaise, onset of arthritis, development of severe lesions of the pinnae, and scheduled culls to provide frozen tissue. Persistent fungal infection was ruled out in six severely affected cases by standard histopathological procedures (data not shown). None of the heterozygote or wild-type mice was affected, although two had mild epidermal thickening without peg formation. The mean age of the *Il1rn*−/− mice was less than the controls. Of *Il1rn*−/− mice, 21 of 33 (64%) were judged to be affected. The earliest cases of ear reddening appeared at 54 and 59 d. We detected no significant sex-related difference in susceptibility. *Il1rn*−/− mice that were affected at the time of culling were significantly older ($p < 0.01$, Wilcoxon rank test) than unaffected *Il1rn*−/− mice. The onset of inflammation of the ear pinna was sporadic. Incidence seemed to rise sharply after 100 d age, to affect 20/26 (77%) of older mice.

Cellular composition The dermal infiltrate in human psoriasis is rich in MΦ and $CD4^+$ T cells, during the active phase of the disease, switching to $CD8^+$ during resolution of a plaque (reviewed by Barker and Fry, 1992). $CD4^+$ T cells also infiltrate the epidermis and dendritic cells (DC) are abundant. In wild-type tissue, infrequent $CD4^+$ cells were restricted to the dermis (Fig 2*c*). In affected *Il1rn*−/− mice, $CD3^+$ (not shown) and $CD4^+$ cells were abundant in the

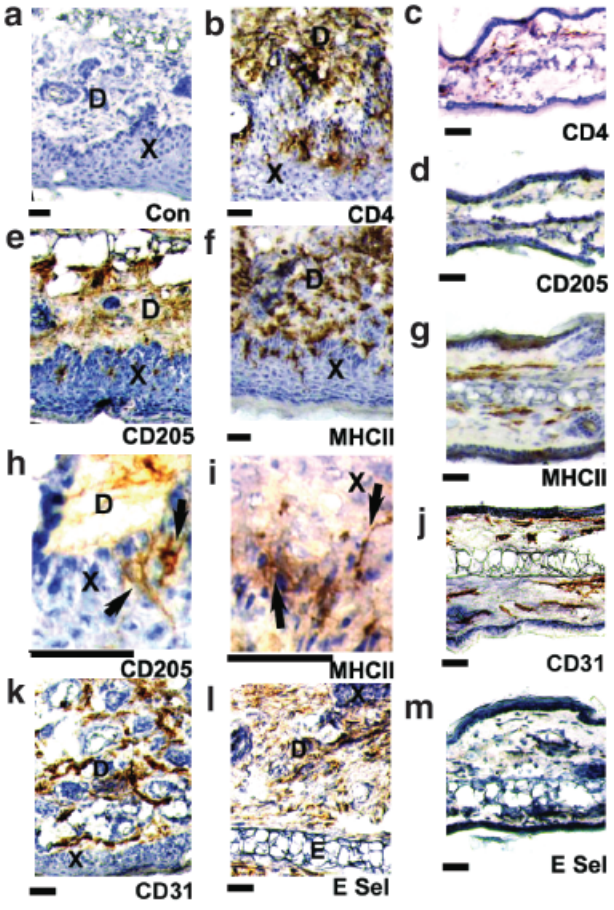

Figure 2
Immunodetection of CD4, DC, and vascular markers in ear pinnae. Scale bars are 50 μm. Antigen is visualized as the brown pigment. Cells were counterstained with hematoxylin. Labels: as in Fig 1. Panels *a*, *b*, *e*, *f*, *h*, *i*, *k* and *l* are from affected *Il1rn*−/− mice. Panels *c*, *d*, *g*, *j*, and *m* are taken from wild-type mice. Antibody specificity was as follows; mouse identities are in italic. (*a*) Lacked primary antibody (155 d male, *ko1*). (*b*) CD4 (*ko1*). (*c*) CD4 (171 d male, *wt1*). (*d*) CD205 (DC: *wt1*). (*e*) CD205 (*ko1*). (*f*) H-2IA^d (MHC Class II: 224 d female, *ko2*). (*g*) H-2IA^d (171 d male, *wt2*). (*h*, *i*) As (*e*), (*f*) respectively, arrows indicate branched cells. (*j*) CD31 (191 d female, *wt3*). (*k*) CD31 (*ko1*). (*l*) E-selectin (*ko1*). (*m*) E-selectin (191 d female, *wt4*).

psoriasiform epidermis and dermis (Fig 2*b*). $CD8^+$ cells were infrequent (data not shown). $CD205^+$ or $MHCII^+$ DC were found in similar affected sections (Fig 2*e*, *f*), but were sparse in wild-type tissue (Fig 2*d*, *g*). The epidermal DC (Langerhans cells) have the classic branched morphology of the immature phenotype (Fig 2*h*, *i*). In immature DC, MHC class II is expected to be intracellular but abundant.

Neo-angiogenesis, accompanying dermal thickening, occurs in human psoriasis (Creamer *et al*, 1997), causing the dermal capillaries to become more tortuous, dilated and leaky, and the tissue to appear red (Mordovstev and Albanova, 1989). The mouse model also showed extensive, apparently disorganized expression of the constitutive endothelial marker CD31 (Fig 2*k*) compared with wild-type specimens (Fig 2*j*). E-selectin (an activation marker) was abundant in affected *Il1rn−/−* tissue (Fig 2*l*) but was seldom observed in wild-type tissue (Fig 2*m*).

Both the infiltrate and the structural cells of human psoriatic epidermis and dermis produce IL-1 (Kristensen *et al*, 1992). IL-1 can initiate most of the inflammatory cellular events seen in psoriasis, and of particular interest is its ability to promote proliferation and to delay differentiation in keratinocytes. A direct role for IL-1 in psoriasis has also been suggested by experiments with K14-IL-1α transgenic mice, which exhibited scaly and erythematous inflammatory skin lesions (Groves *et al*, 1995). IL-1β expression is abundant in the dermis of affected *Il1rn−/−* mice (Fig 3*b*). Some localized expression in wild-type mice (Fig 3*d*) might reflect a response to environmental insult. The presence of IL-1β expression in affected *Il1rn−/−* samples correlated with MΦ (Fig 3*c*). Infrequent MΦ were seen in the dermis of wild-type animals (Fig 3*g*). Lesions were positive for T cell cytokines IFN-γ (Fig 3*e*), IL-4 (Fig 3*f*), and IL-5 (Fig 3*h*), indicating that T_H1 and T_H2 cells are activated. Few IFN-γ-containing cells also appeared in the dermis of wild-type controls (Fig 3*j*). IL-4^+ or IL-5^+ cells were absent from wild type skin (Fig 3*m, k*). The presence of activated T cells, particularly T_H1 $CD4^+$, is a feature of human psoriasis, but it has been shown recently that the class responsible for the adoptive transfer of psoriasis to engrafted human skin is a subset of NK-T cells (Nickoloff *et al*, 1999).

Keratin 6 is an important structural component of the immature keratinocyte and is normally restricted to the hair follicle sheath and the basal layer of injured skin, but is induced *in vivo* and *in vitro* by IL-1 and is expressed throughout the psoriatic suprabasal epidermis (Komine *et al*, 2001). In human psoriasis, broad keratin 6 expression has been interpreted as indicative of keratinocyte activation (Stoler *et al*, 1988). In the mouse, staining in the wild-type tissue was similarly restricted (Fig 3*l*), in agreement with previous studies (Stoler *et al*, 1988). Fig 3*i* shows anti-keratin 6 staining of affected *Il1rn−/−* tissue. Expression extended throughout the expanded subcorneal layers.

Incidence of cutaneous inflammation: Overlap of inflammatory phenotypes in *Il1rn−/−* BALB/c *Il1rn−/−* BALB/c (Harlan), like BALB/cA (Horai *et al*, 2000), are also susceptible to both rheumatoid-like arthritis and large vessel arteritis (Nicklin and Iwakura, unpublished results, see also Table I). A subset of 27 *Il1rn−/−* BALB/c from the group described above had been examined while alive for joint swelling, which indicated advanced arthritis, and were also examined histologically for arteritis at the aortic root, a favored site in *Il1rn−/−* BALB/c. The incidence of inflammation of the joints, the ear pinna, and the aortic root was found to be 19 of 27, 20 of 27, and 21 of 27, respectively. However, the overlap between the three diseases was not significantly different from random association.

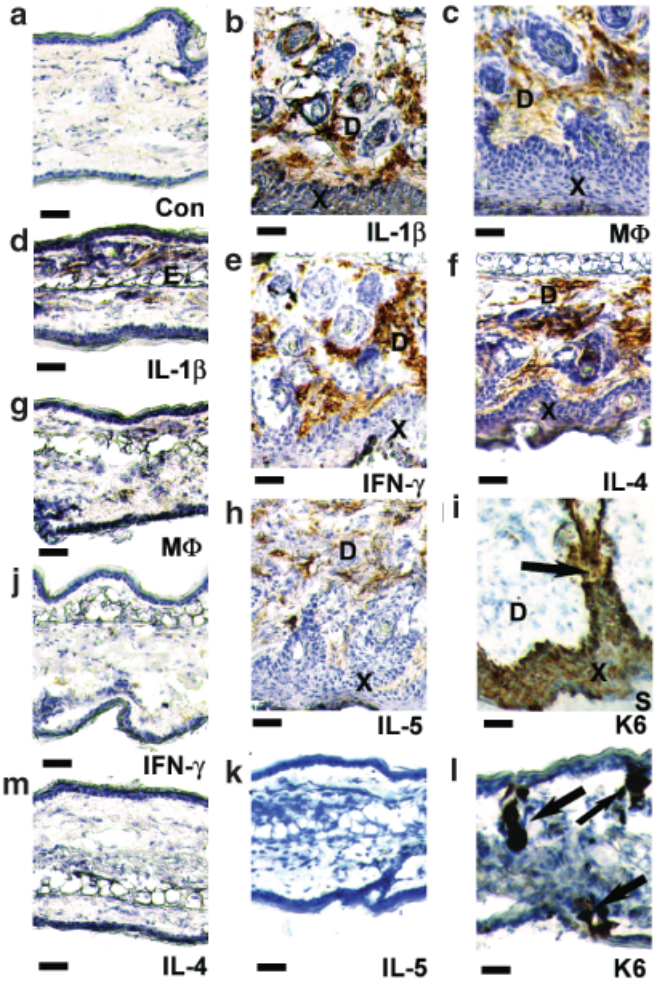

Figure 3
Immunodetection of macrophages, cytokines, and keratin 6 in ear pinnae. Scale bars are 50 μm. Antigen is indicated by the brown pigment. Cells were counterstained with hematoxylin. Labels: as in Fig 1. Tissue in panels *b*, *c*, *e*, *f*, *h,* and *i* are from affected *Il1rn−/−* mice. Panels *a*, *d*, *g*, *j*, *k*, *l*, and *m*, wild-type mice. Antibody specificity was as follows; mouse identities are in italic. (*a*) Lacked primary antibody (*wt1*). (*b*) IL-1β (*ko1*). (*c*) F4/80 (MΦ: *ko1*). (*d*) IL-1β (*wt4*). (*e*) IFN-γ (*ko1*). (*f*) IL-4 (*ko1*). (*g*) F4/80 (*wt4*). (*h*) IL-5 (*ko1*). (*i*) Keratin-6 (137 d male, *ko3*), arrow indicates collar of hair follicle. (*j*) IFN-γ (*wt4*). (*k*) IL-5 (*wt4*). (*l*) keratin-6 (*wt2*), arrows indicate follicle sheaths. (*m*) IL-4 (*wt3*).

Evidence for genetic control of susceptibility Data on the strain-specific incidence of the three diseases are collated in Table I. In our laboratory, Sf *Il1rn−/−* (Nicklin *et al*, 2000) develop arteritis but not arthritis or cutaneous inflammation, while C57BL/6 *Il1rn−/−* have shown no propensity to develop any of the diseases, as we have assayed them. However, five of seven archived *Il1rn−/−* Sf × BALB/c F2 mice developed cutaneous inflammation. Horai *et al* (2000) have reported that of the strains they tested, only BALB/cA *Il1rn−/−* were susceptible to arthritis

Table I. Frequency of ear skin lesions, arthritis, and aortitis in *Il1rn*−/− mice according to genetic background

Strain	*H-2* haplotypes	Median age (d)	Incidence of inflammation		
			Cutaneous	Joint[a]	Aorta
Sf (colony)[b]	*b/u*	170	0/55[c]	0/55	49/55[b]
Sf × BALB/c F2	ND	211	5/7[d]	0/7	⩾2/7
BALB/c[e]	*dd*	147	19/27[d]	20/27	21/27
C57BL/6	*bb*	202	0/18[d]	0/18	0/18
BALB/c × C57BL/6 F1	*bd*	200	0/12[d]	0/12	2/12
BALB/c × C57BL/6 F2	*b/d*	200	0/95[c]	7/95	6/14

[a]Joint inflammation was identified visually and was recorded where a limb showed swelling.
[b]Data taken from Nicklin *et al* (2000).
[c,d]Cutaneous inflammation of the ear pinnae was determined visually[c] or by histology[d]. Only a histological score of ⩾2 is recorded as positive.
[e]This set of mice is a subset of the group described in Results that were examined for cutaneous lesions.

when IL-1ra deficient. Extending these observations here, we reared 12 F1 and 95 F2 from an *Il1rn*−/− BALB/c × C57BL/6 cross. At 200 days, aortitis was common in F2, whereas arthritis was rare and cutaneous inflammation was absent. These results strongly suggest that there are some genetic loci, which do not overlap, that determine susceptibility to the three diseases. Starting with a larger cohort of F2 *Il1rn*−/− BALB/c × C57BL/6, it will be possible to map these loci.

The role of IL-1ra deficiency Although the deficiency of IL-1ra underlies each phenotype, the point at which it is involved in each disease is not easily defined. The role of IL-1ra in human psoriasis is not clear, since both IL-1 and IL-1ra are reported to be expressed in both the cells of the infiltrate and in the epidermis. We have no information yet on the events that initiate the disease. Our current studies, like those of human inflammatory diseases, are of the secondary chronic phase, which has the hallmarks of a well-established T cell-mediated response, with cytokine-containing T cells present, as has been suggested in human psoriasis (Valdimarsson *et al*, 1995). Chronic inflammation might be sustained by a quite different cytokine network from the one that initiates it.

Psoriasis does not occur without intervention in any model animal species. Genetic alteration by transgenesis, targeted mutation, and the study of serendipitous mutants has resulted in several mouse models of psoriasis-like dermatoses. Mice manipulated by the transplantation of human skin have also been highly informative in understanding the disease process (Nickoloff *et al*, 1999). Unlike most models, deficiency of the *Il1rn* gene creates a phenotype that is spatially predictable and limited (for unknown reasons), yet within the lesions, appears to have a full set of features of the human disease. It may therefore prove to be a useful experimental model.

Materials and Methods

Mice *Il1rn*-deficient mice have been described previously (Nicklin *et al*, 2000). Inbred "Sheffield" lines (Sf), derived from the original 129 × MF1 litters, were re-derived into specific pathogen-free conditions and crossed with BALB/c (Harlan). All mice were housed behind a positive pressure barrier where all materials were supplied sterile. Mice were periodically sampled and found to be seronegative for specific pathogens (Nicklin *et al*, 2000). They were reared under appropriate UK Home Office Licenses. The work was approved by the ethical review panel of the University of Sheffield.

Archived material Body cavities were opened and mice were stored in 4% formaldehyde in phosphate buffer. Ear pinnae were processed to paraffin by standard procedures. Transverse sections were cut 6 μm thick. Neutrophil specific esterase (chloroacetate esterase) was detected in sections with a reagent kit from Sigma Chemical Corporation (Poole, UK), according to the manufacturer's instructions. Mast cells were stained with toluidine blue.

Immunohistology Ear pinnae were removed from freshly killed mice and were snap frozen in liquid nitrogen. Standard techniques were used, with the exception that for uniformity, saponin (1 g per liter) was used in all incubations and washes, except for the detection of H-2IA[d]. After brief acetone fixation, endogenous peroxidase was blocked with 0.25% H_2O_2 for 20 min. Non-specific antibody was blocked by incubation with 7% appropriate serum. Endogenous avidin was saturated using reagents from Vector Labs (Peterborough, UK) Primary antibodies were commercially available monoclonal rat anti-mouse, except for rabbit polyclonal anti-keratin 6 antibody. A list is available from the authors on request. Primary rat and rabbit antibodies were detected with biotinylated rabbit anti-rat IgG (Vector Labs) and biotinylated goat anti-rabbit IgG, respectively (Vector Labs). Bound secondary antibodies were detected with peroxidase-labeled avidin–biotin complexes (ABC reagents: Vector Labs). The final brown pigment was produced by peroxidation of benzamidine. Results were accepted if parallel reactions without primary antibody gave no detectable staining.

We thank Orla Gallagher for advice and technical assistance, Janine Timms for technical assistance, and the staff of the Field Laboratories for animal husbandry. We thank Dr Andrew McDonagh and Dr David Hughes for discussions of the histopathology. This work was supported under Contract QLRT 1999-02072 under the European Union Framework V, Quality of Life Programme.

DOI: 10.1111/j.0022-202X.2004.22305.x

Manuscript received March 20, 2003; revised September 5, 2003; accepted for publication September 5, 2003

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