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(21) International Application Number: PCT/GB96/01930 (22) International Filing Date: 8 August 1996 (08.08.96) (30) Priority Data: 9516287.1 9 August 1995 (09.08.95) GB (71) Applicant (for all designated States except US): THE VICTORIA UNIVERSITY OF MANCHESTER [GB/GB]; Oxford Road, Manchester M13 9PL (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): FERGUSON, Mark, William, James [GB/GB]; 13 Peelmoat Road, Heaton Moor, Stockport, Cheshire SK4 4PL (GB). (74) Agents: McNEIGHT, David, Leslie et al.; McNeight & Lawrence, Regent House, Heaton Lane, Stockport, Cheshire SK4 1BS (GB).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: PHARMACEUTICAL COMPOSITION CONTAINING IL-10 (57) Abstract The present invention provides IL-10 or a fragment or a partially modified form thereof for use in promoting the healing of wounds and fibrotic disorders with reduced scarring and methods for same.		

PHARMACEUTICAL COMPOSITION CONTAINING IL-10

The present invention concerns pharmaceutical compositions for promoting the healing of wounds or fibrotic disorders, in particular for promoting the healing of wounds or fibrotic disorders with reduced scarring.

By "wounds or fibrotic disorders" is meant any condition which may result in the formation of scar tissue. In particular, this includes the healing of skin wounds, the repair of tendon damage, the healing of crush injuries, the healing of wounds to the eye, including wounds to the cornea, the healing of central nervous system (CNS) injuries, conditions which result in the formation of scar tissue in the CNS, scar tissue formation resulting from strokes, and tissue adhesion, for example, as a result of injury or surgery (this may apply to e.g. tendon healing and abdominal strictures and adhesions). Examples of fibrotic disorders include pulmonary fibrosis, glomerulonephritis, cirrhosis of the liver, systemic sclerosis, scleroderma and proliferative vitreoretinopathy.

By "reduced scarring" is meant reduced level of scarring relative to an untreated wound or fibrotic disorder.

In particular, there is a lack of compositions for promoting the healing of wounds or fibrotic disorders with reduced scarring. Scar tissue formation, although providing mechanical strength to a healed wound, can be unsightly and may impair the function of the tissue.

This is particularly the case in wounds which result in scar tissue formation in the CNS, the scar tissue inhibiting the reconnection of severed or re-growing nerve ends, so significantly affecting their function.

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There is also a lack of compositions for treating and promoting the healing of chronic wounds, for example venous ulcers, diabetic ulcers and bed sores (decubitus ulcers), especially in the elderly and wheel chair bound patients. Such compositions may be extremely useful in patients where wound healing is either slow or in whom the wound healing process has not yet started. Such compositions may be used to "kick-start" wound healing and may then be used in combination with compositions for promoting healing with reduced scarring. Hence not only may a chronic wound be healed, but it may be healed with reduced scarring.

IL-10 (Interleukin-10) was originally identified as a product of Th2 cells (Fiorentino, D.F. and Moddman, T.R., 1989, *J. Exp. Med.*, 170: 2081-2095) but was also independently identified (O'Garra, A. *et al.*, 1990, *Internal Immunol.*, 2: 821-823) as a product of B - cell lymphomas that prolonged the survival of mast cells and enhanced proliferation of thymocytes.

Molecular characterisation of human and murine IL-10 by Moore, K.W. *et al.* (1990, *Science*, 248: 1230-1234) and Vieira, P. *et al.* (1991, *Proc. Natl. Acad. Sci. USA*, 88: 1172-1176) showed that there was an 80% homology of their nucleotide sequences. Mouse IL-10 (mIL-10) protein consists of 157 amino acids with two potential N- glycosylation sites although glycosylation is not essential for the biological activities of mIL-10. Human IL-10 (hIL-10) protein consists of 160 amino acids with one potential N-glycosylation site which is not used (Vieira *et al.*, 1991). Both mIL-10 and hIL-10 contain four cysteine residues that form two intramolecular disulphide bonds generating biologically active homodimers with molecular weights of 32 kDa and 39 kDa respectively, and it is not clear whether monomeric forms of IL-10 are biologically active. Although there is 80% homology between hIL-10 and mIL-10, only hIL-10 acts on both human and mouse cells, whereas mIL-10 has species specificity activity (Vieira *et al.*, 1991; Kim, J.M. *et al.*, 1992, *J. Immunol.*, 148: 3618-3623).

There are many cellular sources and major biological activities of IL-10, all of which may play some role in the wound microenvironment. It has been shown that IL-10 possesses many stimulatory and inhibitory effects - van Vlasselar *et al.* (1994, J. Cell Biol., 124: 569-577) showed that IL-10 inhibited TGF- β synthesis required for osteogenic commitment of mouse bone marrow cells, and hence the resulting mineralised matrix, whereas Go *et al.* (1990, J. Exp. Med., 172: 1625-1631) showed IL-10 to be a novel B-cell stimulatory factor. IL-10 has also been shown by Bogdan *et al.* (1991, J. Exp. Med., 174: 1549-1555) to directly act on macrophages and inhibit their subsequent activation and hence release of pro-inflammatory cytokines (see also Berg. D. J. *et al.*, 1995, J. Exp. Med., 182: 99-10; Chernoff, A. E. *et al.*, 1995, J. Immunol. 154 (10): 5492-5499).

Despite the aforementioned studies of cytokines, the present inventor has found that, surprisingly, IL-10 may be used to promote the healing of wounds or fibrotic disorders with reduced scarring. It appears that by inhibiting inflammation at a wound site or site of a fibrotic disorder, in particular at an early stage after wounding/onset, there is a "knock-on" effect upon the resulting collagen matrix, resulting in an improved architecture and reduced scarring. This result is particularly surprising since in the short-term, there was no inhibition of re-epithelialisation or early wound repair, whilst in the longer-term, it improved the quality of later scar formation and reduced scarring.

In a first aspect, the invention provides a use of IL-10 or a fragment or a partially modified form thereof for promoting the healing of a wound or a fibrotic disorder, the use characterised in that the healed wound or healed fibrotic disorder has reduced scarring.



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By "fragment or partially modified form thereof" is meant a fragment or partially modified form of IL-10 which
5 retains the anti-inflammatory healing functionality of IL-10, although it may of course have additional functionality. Partial modification may, for example, be by way of addition, deletion or substitution of amino acid residues.

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For example, a substitution may be conserved substitution. Hence the partially modified molecules may be homologues of IL-10. They may, for example, have at least 40% homology with IL-10. They may for example have
5 at least 50, 60, 70, 80, 90 or 95% homology with IL-10.

IL-10 or a fragment or a partially modified form thereof may be for use in conjunction with a pharmaceutically acceptable carrier, diluent or excipient.

IL-10 or a fragment or a partially modified form thereof may be for use in conjunction with a composition for promoting the healing of wounds of fibrotic disorders with reduced scarring.

IL-10 or a fragment or a partially modified form thereof may be for use in conjunction with a composition for promoting the healing of chronic wounds.

In a second aspect the invention provides a method of promoting the healing of a wound or a fibrotic disorder comprising, contacting the wound or fibrotic disorder with
5 IL-10 or a fragment or a partially modified form thereof, so that the healed wound or healed fibrotic disorder has reduced scarring.

IL-10 or a fragment or a partially modified form
10 thereof may be administered to a wound site or site of a fibrotic disorder.

IL-10 or a fragment or a partially modified form thereof may be administered at a concentration of between
15 about 1 μ M and about 10 μ M. It may be administered at a concentration of between about 2.5 μ M and about 5 μ M.

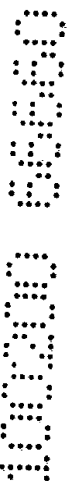


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IL-10 or a fragment or a partially modified form
5 thereof may be administered immediately prior to wound
healing, but may be effective if administered within about
7 days of wounding. It could be administered on at least
two occasions.

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The method may be used in conjunction with a method or composition for promoting the healing of wounds or fibrotic disorders with reduced scarring.

The method may be used in conjunction with a method or composition for promoting the healing of chronic wounds.

The invention will be further apparent from the following example, with reference to the several figures of the accompanying drawings, which shows, by way of example only, compositions and methods of promoting the healing of wounds or fibrotic disorders with reduced scarring.

Of the figures:

Figure 1 shows the inflammatory profile of incisional wounds treated with IL-10, injected at day 0;

Figure 2 shows the inflammatory profile of incisional wounds treated with IL-10, injected at days 0 and 7;

Figure 3 shows the blood vessel profile of incisional wounds treated with IL-10, injected at day 0; and

Figure 4 shows the blood vessel profile of incisional wounds treated with IL-10, injected at days 0 and 7.

In the claims which follow and in the preceding summary of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprising" is used in the sense of "including", i.e. the features specified may be associated with further features in the various embodiments of the invention.



EXAMPLES
EXPERIMENTAL

Rats were wounded and treated with various compositions and then harvested and the wounds analysed in order to analyse the effects of anti-inflammatory cytokines upon wound healing. Results show that in the short-term, there was no inhibition of re-epithelialisation or early wound repair, whilst in the longer-term, it improves the quality of later scar formation and reduced scarring.

Material and Methods

Male Sprague Dawley rats age and weight matched at 200-250 g were anaesthetised using equal parts halothane, oxygen and nitrous oxide. 1 cm full thickness (through the panniculus carnosus) linear incisions were made 5 and 8 cm from the base of the skull and 1 cm either side of the dorsal midline. The wounds were treated by intradermal injection with either 100 μ l of IL-10 (2.5 μ g/ml) (Genzyme) or phosphate buffered saline (PBS) for control. Animals were split into three groups: group A were injected with IL-10 or PBS on day 0 prior to wounding, group B were injected with IL-10 or PBS on day 0 prior to wounding and day 7 post wounding (pw). A third group (C) had the same injection regime as group B although they were treated with double the dose of IL-10 (5 μ g/ml). Animals were killed on days 3 (group A only), 7, 14 and 84 post wounding. Wounds and approximately 0.5 cm of normal skin either side, were excised and bisected for routine wax histology and immunocytochemistry.

A further group of eight animals were injected with 100 μ l of IL-10 (1.25 μ g/ml) on days 0 and 7 only. Animals were killed on 7 and 84 days post wounding. After macroscopic analysis wounds were excised for routine histology and immunocytochemistry as before. A repeat group of eight animals were injected with 100 μ l of IL-10 (2.5 μ g/ml) and killed at 84 days post wounding. After macroscopic analysis wounds were excised and treated as before.



7 µm-thick wax sections were cut and stained with Haemotoxylin and eosin, Mallory's and Masson's collagen trichrome stain for the assessment of cellular infiltrate and collagen architecture respectively and Gomori aldehyde fushin stain for elastin. 7 µm-thick cryosections were cut and stained with antibodies to assess inflammation (ED1; Serotec), angiogenesis (von Willebrand factor) and extracellular matrix deposition (fibronectin and collagen I). Wound sections were analysed in detail using a Joyce Lobel image analysis Magiscan. Six areas, within the wound margins below the epidermal/dermal junction and above the dermal/panniculus junction, were viewed through a x10 objective and images were captured and using the analysis package GENIAS 25 (Joyce Lobel) the area stained within the field was obtained. Results are collated and presented as means and standard errors (Figures 1 to 4).

Results

Macroscopic

Macroscopic appearances of treated and control wounds were captured using a PC image analysis system. The wounds were scored on a linear scale from 0-5 with 0 being normal dermis and 5 a bad scar. 90% of treated wounds score 2 or less, whereas 10% were in the 3 and 4 bracket. 90% of control wounds scored 3 or more and 10% scored 2 or less. Macroscopically there appears to be less scar formation with treatment of IL-10 compared to controls.

Histology

Qualitative analysis of H&E (Haemotoxylin and eosin) stained wound sections shows that IL-10 treatment decrease the number of inflammatory cells influxing into the wound at day 3 and 7 post wounding when compared to PBS treatment (control). The degree of scarring is qualitatively assessed by studying Masson's trichrome stained wound sections at 84 days post wounding and grading features of the neodermis such as fibre size, length and density. Wounds treated with IL-10 (2.5 µg/ml) on day 0 only show

improved restitution of the dermal architecture when compared with control wounds. The IL-10 treated wounds have larger, less densely packed fibres in a more random orientation (basket weave) compared with control wounds where the collagen fibres are finer, more densely packed and aligned parallel to the epidermis. When wounds are treated on day 0 and day 7 with IL-10 (2.5 µg/ml), the resultant dermal architecture resembles normal skin with a more basket weave configuration of the collagen fibres within the wound. The appearance of the scar is far superior to control wounds and wounds treated with IL-10 on day 0 only. 2.5 µg/ml of IL-10 appears to be the maximal dose as wounds treated with the higher dose (5 µg/ml) have a more visible macroscopic scar. Elastin architecture was assessed using Gomori aldehyde fushin stain. In early control or treated wounds there was little elastin staining when compared to normal dermis but at 84 days although there were fewer fibres in wounds compared to normal dermis there was an increase in elastin staining in IL-10 treated wounds compared to controls. The elastin fibres were associated with the collagen fibres in the scar. Whilst IL-10 treatment appears to inhibit inflammation and improve the quality of later scar formation, it does not inhibit re-epithelialisation or early wound repair.

Immunocytochemistry

Qualitative histological analysis was further corroborated by quantitative image analysis which shows that IL-10 inhibits the infiltration of monocytes and macrophages into the wound when compared to controls (Figures 1 and 2), although IL-10 has no effect on angiogenesis within the wound when compared to controls (Figures 3 and 4). Staining for fibronectin shows that IL-10 treated wounds have less fibronectin present in the wound area at 3 and 7 days when compared with control wounds. Immunostaining for transforming growth factor beta 1 (TGFβ₁) showed little differences in cellular staining (mainly monocytic) between control and treated wounds although there were fewer cells in the IL-10 treated wounds.

Tables 1-4 (below) show the results contained in Figures 1-4 respectively. Results are given as area stained (μm^2) x100, followed by the standard error of the mean in brackets (n=4). Results given as zero indicate that there was no detectable staining.

Table 1: (Figure 1) Inflammatory cell (ED1) profile of incisional wounds treated with IL-10 (injected at day 0)

	IL-10	Control
3 days pw	15.269 (1.578)	51.004 (2.246)
7 days pw	0.321 (0)	71.704 (3.384)

Table 2: (Figure 2) Inflammatory cell profile of incisional wounds treated with IL-10 (injected at days 0 and 7)

	IL-10	Control
7 days pw	3.123 (1.109)	71.704 (3.384)
14 days pw	0	5.041 (0.697)

Table 3: (Figure 3) Blood vessel profile of incisional wounds treated with IL-10 (Injected at day 0)

	IL-10	Control
3 days pw	20.456 (1.855)	18.118 (1.700)
7 days pw	1.355 (0.719)	4.368 (0.712)
14 days pw	0	4.432 (0.948)

Table 4: (Figure 4) Blood vessel profile of incisional wounds treated with IL-10
(injected at days 0 and 7)

	IL-10	Control
7 days pw	5.128 (0.069)	4.368 (0.712)
14 days pw	0	4.432 (0.948)

CLAIMS

1. A use of IL-10 or a fragment or a partially
5 modified form thereof for promoting the healing of a
wound or a fibrotic disorder, the use characterised
in that the healed wound or healed fibrotic disorder
has reduced scarring.
2. A use of IL-10 or a fragment or a
10 partially modified form thereof according to claim 1
in conjunction with a pharmaceutically acceptable
carrier, diluent or excipient.
3. A use of IL-10 or a fragment or a partially
15 modified form thereof according to any one of the
preceding claims in conjunction with a composition
for promoting the healing of wounds with reduced
scarring.
4. A use of IL-10 or a fragment or a partially
20 modified form thereof according to any one of the
preceding claims in conjunction with a composition
for promoting the healing of chronic wounds.
5. A method of promoting the healing of a wound or
a fibrotic disorder comprising, contacting the wound
or fibrotic disorder with IL-10 or a fragment or a
25 partially modified form thereof, so that the healed
wound or healed fibrotic disorder has reduced
scarring.
6. A method according to claim 5, IL-10 being
administered to a wound site or site of a fibrotic
30 disorder.



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7. A method according to claim 6, wherein IL-10 or a fragment or a partially modified form thereof being administered at a concentration of between about 1 μ M and 10 μ M.

8. A method according to claim 7, IL-10 or a fragment or partially modified form thereof being administered at a concentration of between about 2.5 μ M and about 5 μ M.

9. A method according to any one of claims 5-8 for use in conjunction with a method for promoting the healing of wounds or fibrotic disorder with reduced scarring.

10. A method according to any one of claims 5-9 for use in conjunction with a method for promoting the healing of chronic wounds.

11. A use according to claim 1, substantially as described herein with reference to the accompanying Examples.

12. A method according to claim 5, substantially as described herein with reference to the accompanying Examples.

DATED THIS DAY 10 February 2000

THE VICTORIA UNIVERSITY OF MANCHESTER

By their Patent Attorneys

GRIFFITH HACK



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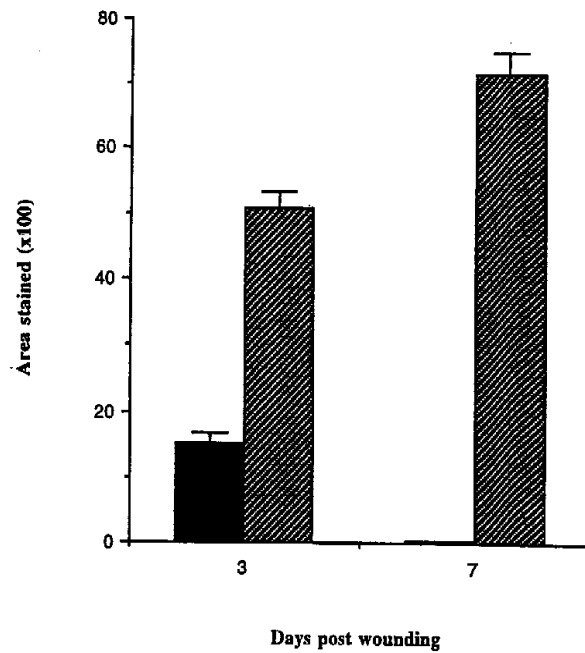


FIGURE 1

Inflammatory cell
profile of incisional
wounds treated with
IL-10 (0 inj)

Inflammatory cell profile of incisional wounds treated with IL-10 (0,7 inj)

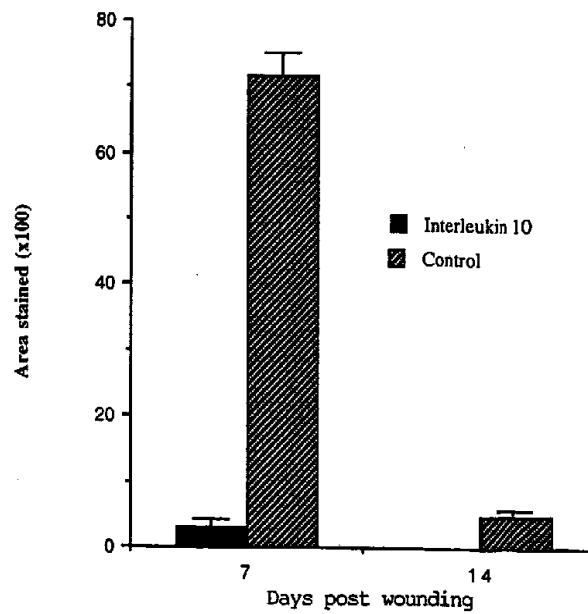


FIGURE 2

Figure 1 and 2: Inflammatory cell profile (ED1) assessed using image analysis.

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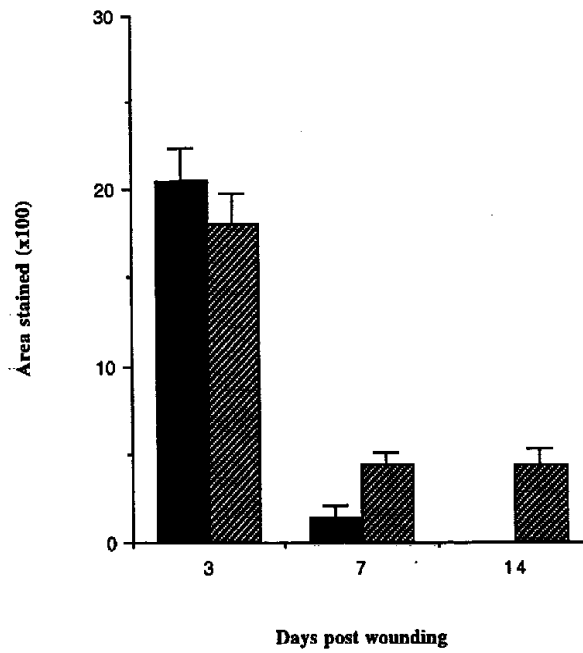


FIGURE 3

Blood vessel
profile of
incisional wounds
treated with
IL-10 (0 inj)

Blood vessel profile of incisional wounds treated with IL-10 (0,7 inj)

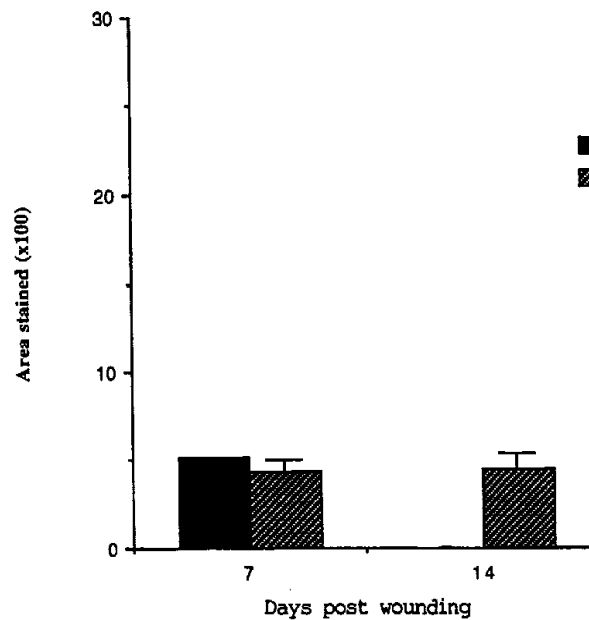


FIGURE 4

■ Interleukin 10
▨ Control

Figure 3 and 4: Blood vessel profile (von Willebrand factor) assessed using image analysis.

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