INVESTIGATIVE REPORT

Sterile Trauma to Normal Human Dermis Invariably Induces IL1ß, IL6 and IL8 in an Innate Response to "Danger"

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Microdialysis allows the study of the local production and temporal resolution of cytokines in living skin. Samples were taken from the normal skin of 10 healthy subjects for 24–28 h after insertion of a concentric microdialysis catheter, and analysed with a Luminex bead-based assay. Interleukin-1 beta (IL1b), IL6 and IL8 were seen in all subjects at all time-points after the first hour. Levels peaked at 5–8 h, equilibrating to lower levels at 24 h. Immunohistological double staining for human leukocyte antigen (HLA)-DR and intracellular cytokines on biopsies taken after catheter removal showed many stained cells in the dermis, in contrast to the few cells stained in the epidermis. This study demonstrates the reactive capability of the dermis when provoked separately from the epidermis. The production of IL1b, IL6 and IL8 occurs invariably in what can be termed an innate, dermal response to "danger"; in this case in the form of sterile needle trauma. *Key words: microdialysis; human; dermis; cytokines; chronology; innate immunity; bead immunoassay.*

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Cutaneous microdialysis enables continuous *in vivo* sampling of the dermal interstitial fluid in clinical monitoring and in the study of processes of metabolic, pharmacokinetic, pharmaco-dynamic and inflammatory relevance (1–3). Measurement of cytokines is also possible (1, 4), and the use of bead-based immunoassays has increased the number of cytokines that can be estimated simultaneously in an individual sample (5). Together, these techniques have been used recently to profile *in vivo* inflammatory reactions (6–8).

For the interpretation of microdialysis studies in diseased skin, issues around the interpretation of the findings from uninvolved (healthy) skin remain to be fully elucidated (9). The skin's response to the trauma caused during insertion of the catheter includes disruption of the tissue architecture, and direct damage to cells and capillaries, leading to secretion and production of inflammatory mediators (10–12). The establishment

of an equilibration time after catheter insertion is an important issue. There is wide agreement that in most situations 30–135 min is the time required for the tissue to return to "normal" in most aspects of its function (3, 11, 13). Whether this time is adequate in the study of cytokines has not been established, much less studied in detail. Based on previous findings on induction of interleukin (IL)-6 by catheter insertion (4), it has been assumed that the equilibration time after catheter insertion might be quite long and, accordingly, the present study was conducted over 24 h. Cytokine levels may not return to zero, since the ongoing presence of the catheter in the skin might be expected to give some continuing stimulation.

As well as the above issues, the response of the dermis to the sterile minimal trauma that the catheter insertion constitutes is of interest for the understanding of the reactive capability of the dermis itself. In contrast to the usual experimental situation, in which epidermis and dermis are provoked in parallel (e.g. patch testing, phototesting), the placement of the microdialysis catheter membrane is a purely dermal provocation, the epidermis being involved only at the point of insertion at 1–2 cm distance from the position of the microdialysis membrane itself. Thus, inherent reactive capabilities of the dermis itself can be inferred from responses seen around the membrane area.

The aim of the present study was to investigate in normal forearm skin the release and chronology of pro-inflammatory and T-helper (Th)1/Th2 relevant cytokines (IL-1b, 2, 4, 5, 6, 8, 10, granulocyte-monocyte colony-stimulating factor (GM-CSF), interferon gamma (INFγ), and tumour necrosis factor alpha (TNFα)) hourly over 24 h. The findings are expected to be of relevance to the establishment of an equilibration time after catheter insertion, to the establishment of baseline levels of cytokines, and to the inherent (innate) reactive capability of the dermis to stimulation.

MATERIALS AND METHODS

Subjects

Ten healthy volunteers (4 females and 6 males, age range 27–55 years) were given verbal and written information about the procedure. The study was approved by the Regional Ethics Committee for Human Research at Linköping University (#03250).

Experimental design

Microdialysis catheters used in this study were commercially manufactured CE-marked concentric catheters (CMA71) (CMA/Microdialysis AB, Stockholm, Sweden), as shown in Fig 1a. The catheters have a 10-mm polyethersulfone (PES) membrane with a molecular weight cut-off of 100 kDa. Fig. 1b shows the catheter placed in normal ventral forearm skin. The point of insertion was anaesthetized with a 0.1 ml bubble of local anaesthetic (Xylocain® 10 mg/ml Astra Läkemedel AB, Södertälje, Sweden), injected intradermally. An 18-gauge Venflon venous catheter (Viggo Products, Helsingborg, Sweden) was used as a guide for the microdialysis catheters by tunnelling in the deep dermis or subcutaneous tissue for the first 1.5 cm and then intradermally for the last cm in order to position the dialysis catheter membrane as superficially as possible. Fig. 1c shows the position of the catheter beneath the epidermis. Catheter depth as well as skin (epidermis + dermis) thickness was measured by ultrasound scanning with a Dermascan A (Sonotron AB, Malmö Sweden) (11). Fig. 1c also shows a cross-section of the catheter and the flow of the perfusate, Ringer Dextran Braun 60 (RD60) (Apoketsbolaget, Gothenburg, Sweden) within the catheter. A portable pump (CMA 106, CMA/Microdialysis AB, Stockholm, Sweden) with a fixed flow rate of 0.3 µl/min was used to pump the perfusate through the catheter. The dialysate was collected at 60 min intervals in pre-weighed micro-centrifuge tubes (Elkay Products, MA, USA) for the following 8 h. After the first 8 h, the dialysate was collected in one tube during a 4–7 h period during the evening. The night sample was composed of dialysate collected in one tube while the individual was sleeping. The following morning, the samples were again collected hourly until termination of the experiment 24–26 h after catheter insertion. Prior to the withdrawal of the catheter, the position of the membrane lying in the dermis was marked on the skin surface with a marker pen (see Fig. 4a). The catheter was removed and, after anesthetization with a local anaesthetic, a 4 mm punch biopsy was taken from the area where the membrane had lain within the skin, positioning the marking across the diameter of the circular biopsy. The biopsy, mounted in OCT Compound (Histolab, Gothenburg, Sweden), was positioned prior to freezing so that the skin marking (which was a projection of the probe placement on the skin surface) was perpendicular to the section plane. The tissue was snap frozen in liquid nitrogen and kept at –70°C before processing.

Recovery experiments

The recovery (the amount of cytokines diffusing across the 100 kDa membrane) was assessed under bench top conditions (5). The cytokines (IL-1b, 2, 4, 5, 6, 8, 10, GM-CSF, INFγ, and TNFα) were obtained from the Human Cytokine 10-plex Kit (Biosource, Nivelles, Belgium). Three membranes were perfused at a flow rate of 0.3 µl/min. Samples were collected every hour for 3 h. The *in vitro* recovery for the measured cytokines is expressed as the median ($n = 3$). GM-CSF and IL1b gave the highest percent recovery 21.8% and of 21.6%, respectively, IL5, IL6, IL8 and IFNγ between 11.8% and 18.1%, and IL2, IL4, IL10 and TNFα showed recoveries of 5.3% or less.

Microdialysate

The microdialysate from the 10 individuals was analysed for cytokine content using the Human Cytokine Ten Plex Kit, a method based on multiplex bead technology (14). Briefly, polystyrene beads 5.6 µm in diameter contain a ratio of red and infrared fluorescent dyes particular for a single bead set. Each bead set has been covalently coupled with an antibody directed against one cytokine, in this study IL-1b, 2, 4, 5, 6, 8,
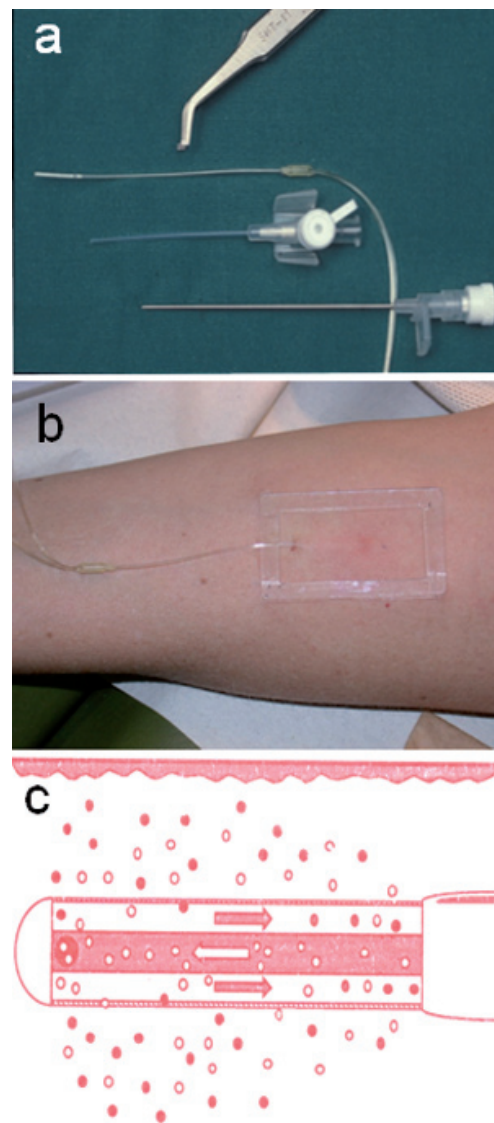

Fig. 1. (a) A concentric microdialysis catheter with a 10 mm membrane lying beside an 18-gauge Venflon venous catheter, which serves as the insertion guide. The stilette lies besides the Venflon. (b) The microdialysis catheter has been inserted into the ventral forearm. The plastic needle of the Venflon (with the wings cut away) has been withdrawn to the outside of the skin, leaving the microdialysis catheter in place within. (c) A cross-section of the microdialysis catheter membrane where the exchange of molecules take place. The perfusion buffer (*arrows*) is pumped into the central tubing, past the membrane where molecules are exchanged and then on towards the collection device.

10, GM-CSF, IFNγ or TNFα. All of these 10 different beads are mixed in a cocktail with the sample. After reacting with the specific cytokine found in the sample, the bead-antibody-cytokine complexes are allowed to react with the detection antibody amplification system directed against the individual cytokines. In the present study the method of the manufacturer was followed except for modifications necessary because of the very low sample volumes (10 µl in this study) inherent to the microdialysis technique. Analysis of the cytokine content of the microdialysate was performed on a Luminex 100 instrument (Biosource, Nivelles, Belgium) a flow cytometer dedicated to measuring Luminex bead-based fluorescence. The instrument was calibrated and validated with Luminex control beads

(Luminex, Austin, TX, USA). The Star Station acquisition program (v2 Applied Cytometry Systems, Sheffield, UK), processes the fluorescence intensity of the beads and bound cytokine-antibody complexes. Standard curves are generated by a five-parameter algorithm in the analysis program and plotted on a log-log scale.

Biopsy

Cryostat sections 10 μm thick from the biopsies of all 10 individuals were first screened using a three-stage immunofluorescence method to detect intracellular presence of the 10 cytokines being studied. The primary antibodies used in this study were monoclonal mouse anti-human IL1b, IL2, IL4, IL5, IL6, IL10, GM-CSF, IFNγ, TNFα and a polyclonal goat anti-human IL8 (R&D Systems, Abingdon, UK), all extensively tested for immunohistochemical use on formalin-fixed cryostat sections. Biotinylated goat anti-mouse immunoglobulin G (IgG) and rabbit anti-goat IgG (DakoCytomation, Stockholm, Sweden) were used as secondary antibodies. To enhance the immunostaining signal of the cytokines, streptavidin conjugated with Alexa Fluor 546 (Molecular Probes, Leiden, The Netherlands) was used as the third step in the staining method. Control of the staining procedure for non-specific antibody staining was done by substituting the primary antibody with isotype and concentration matched non-immune Ig (DAKO Cytomation, Copenhagen, Denmark). Phosphate-buffered saline was also substituted for the primary and secondary antibody. Cryostat sections of a tonsil from a patient with recurrent tonsillitis were used as a positive control (15). A biopsy from normal healthy skin, not subjected to insertion of a microdialysis catheter membrane, was used as a negative control.

New sections from the biopsies demonstrating the presence of any intracellular cytokines were then double-stained, not only for the intracellular cytokines found in the screening, but also for the cell surface antigen human leukocyte antigen (HLA)-DR using a monoclonal mouse anti-human HLA-DR conjugated with fluorescein isothiocyanate (FITC) (DAKO Cytomation, Copenhagen, Denmark). This molecule, expressed on antigen presenting cells, i.e. B-cells, macrophages and Langerhans' cells, can also be expressed on activated keratinocytes (16). The slides were coded and examined in a Nikon confocal laser scanning microscope (LSCM Nikon Eclipse, Nikon, Stockholm, Sweden) by an experienced investigator (FS). Findings will be reported in detail separately, but a summary of the findings comparing the epidermis and the dermis is reported here.

RESULTS

Subjects

Ultrasound measurements showed that the catheters lay at a depth of between 0.5 and 1.1 mm (mean 0.8 mm), which was within or at the limit of the dermis in all subjects. The experiment was successfully completed in all 10 subjects who had engaged in normal daily activities, though not sport or heavy work, with only minor discomfort. Samples were analysable for all periods of collection. The mean % volume recovery (weight of tube after sampling − weight of empty tube before sampling divided by the expected weight/volume × 100) for all samples (n = 131) was 92.5% ± 19.3%. All catheter membranes were visually intact and functioning without leakage after removal from the skin.

Microdialysate

The one hour time resolution of the samples' analytes was achieved in all subjects, as illustrated in Fig. 2 for 3 of the 10 subjects. The sample collected over several hours during the evening (termed "13 h") and the night (termed "20 h") were necessary only for practical reasons (not analytical issues) to do with changing of sampling tubes.

In samples from the first hour after catheter insertion, 26 of 100 possible cytokine findings (10 measured cytokines in 10 subjects) were detected at, or were slightly above, the minimum detectable concentration.

At time-points after the first hour there was a broad production of cytokines with variability between the individual cytokines, individual subjects and time-points. The cytokines seen in all 10 subjects at all time-points were IL1b, IL6 and IL8 (Fig. 3a–c), with the exception of IL1b at 24 h where only 8 of the 10 subjects had detectable concentrations. The box-plot shows the
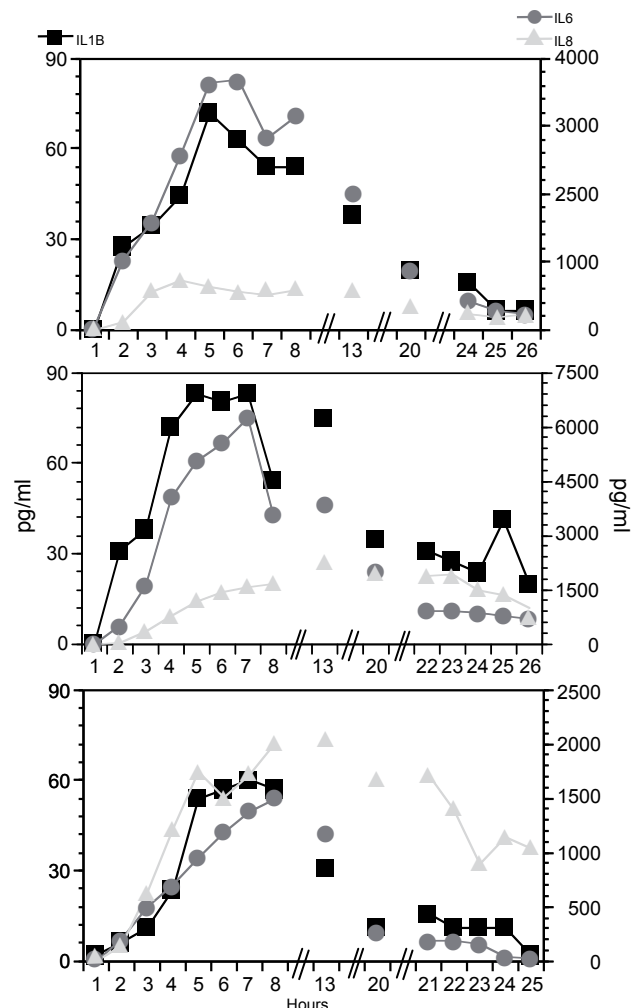

Fig. 2. Representative graphs of interleukin (IL)1b, IL6 and IL8 over the entire experiment for 3 of the 10 individuals (24–26 h). Note the different scales for IL6 and IL8. Samples 13 and 20 h were collected as one sample during the evening and during the night, respectively.

median and upper and lower inter-quartiles. Median values reached a maximal concentration within 7 h and decreased after the night sample ("20 h"), approaching lower and less inter-individually variable values.

Table I illustrates the number of individuals with detectable amounts of cytokines in the microdialysate at four periods during the experiments. The samples collected hourly ("1–8 h") are considered as one collection period, as is the evening ("13 h") and night ("20 h"). The last collection period is the hourly sampling performed before biopsy, termed "22 h-biopsy". The proinflammatory cytokines IL1b, IL6 and IL8 are seen in all 10 individuals in all collection groups. For GM-CSF and TNFα, values were below the lowest detectable concentration at many time-points; however, 8 of 10 and 7 of 10 individuals, respectively, had detectable levels

(not exceeding 40 pg/ml) during the first collection period (1–8 h). Amongst the Th1/Th2 related cytokines, IL2 was the cytokine most commonly found (8 of 10 subjects). IL10 (3/10), IL5 (3/10) and IL4 (1/10) were seldom detected and IFNγ was not detected at all.

Biopsy

In the screening of the biopsy (both epidermis and dermis) for intracellular cytokine expression at 24 h, IL1b was found in 3 of the 10 subjects, IL2 in 4, IL4 in none, IL5 in none, IL6 in 7, IL8 in 9, IL10 in none, GM-CSF in 1, INFγ in 3 and TNFα in 5. Because of the minimal amount of tissue and lack of findings in this first screening, no staining for IL4, 5 and 10 was performed in the following double immunofluorescence staining.

In a detailed study of the epidermis using double immunofluorescence staining, no presence of HLA-DR staining of keratinocytes was detected in any of the 10 individuals. Weak intracellular cytokine staining was seen in a small number of keratinocytes in some of the individuals: IL1b in one of the 3 subjects positive in the intracellular cytokine screening, IL2 in 3 of 4, IL6 in 3 of 7, IL8 in 1 of 9, GM-CSF in 1 of 1, INFγ in 0 of 3 and TNFα in 3 of 5. All individuals, in most sections, showed positive HLA-DR findings in morphologically dendritic cells, presumed to be Langerhans' cells. Some of the dendritic cells present in the epidermis had detectable intracellular cytokines, IL1b in one of 3 and TNFα in 3 of 5 subjects. Fig. 4b–d shows representative pictures from double immunofluorescent-stained sections for the cell membrane antigen HLA-DR and the intracellular
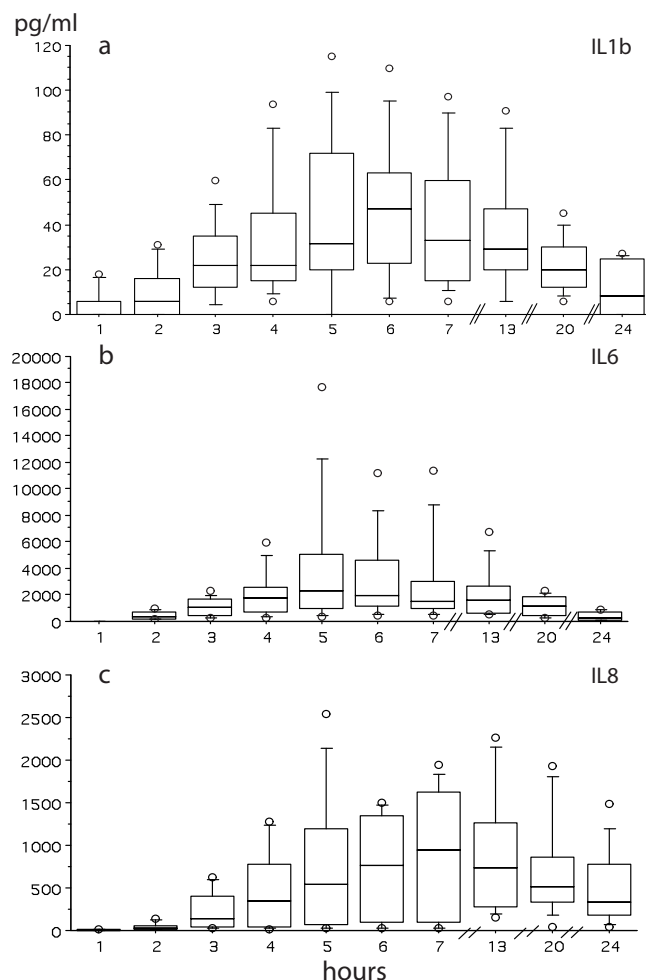

Fig. 3. (a–c) Box-plots showing median and upper and lower interquartiles for (a) interleukin (IL)1b, (b) IL6 and (c) IL8 concentrations in pg/ml from the 10 individuals after insertion of the catheter at time-point 0. Hourly sampling was done for the first 7 h during the day. One sample was collected between 9 and 14 h, during the evening and one sample between 15 and 21 h, during the night. These samples are shown at one time-point, termed "13" and "20" h, respectively, for the sake of clarity. Hourly sampling was continued the following morning until termination of the experiment 24–26 h after catheter insertion. Only those time intervals where samples are available from all 10 individuals are shown.

Table I. *Detectable cytokine in dermal microdialysates in four collection periods. The table shows the samples collected hourly as the first period (termed "1–8 h"), the evening sample (termed "13 h") as the second period, the night sample (termed "20 h") as the third period, and the samples collected hourly for the last 3–6 h before biopsy (termed "22 h-biopsy") as the fourth period. The number of individuals with positive cytokine findings, i.e. cytokines levels above the lowest standard concentration at some time-point during the relevant collection periods is shown*

Cytokine	1–8 h	13 h	20 h	22 h-biopsy
Pro-inflammatory				
IL1b	10	10	10	8
IL6	10	10	10	10
IL8	10	10	10	10
GM-CSF	8	6	5	6
TNFα	7	1	3	4
Th1/Th2				
IL2	6	3	4	8
IL10	3	3	0	1
IL4	0	0	0	1
IL5	1	0	1	3
IFNγ	0	0	0	0

IL: interleukin; IFNγ: interferon gamma; Th1/Th2: t-helper1/t-helper2; TNFα: tumour necrosis factor alpha; GM-CSF: granulocyte-monocyte colony-stimulating factor.

cytokines IL1b, IL6 and IL8, respectively. In contrast to the findings in the epidermis, staining for HLA-DR and cytokines in the dermis was much more pronounced.

DISCUSSION

In this study we have shown the temporal response of cytokines triggered by a sterile needle trauma to the dermis of uninvolved skin. The time resolution of one hour showed 3 cytokines, IL1b, IL6 and IL8, to be invariably induced, being absent or extremely low in the first 1–2 h after insertion and rising to a peak at 3–8 h. Of the remaining cytokines measured, only GM-CSF was seen with any frequency (8 of 10 cases) though at low levels. The samples taken 20 h after insertion showed less variability and lower concentrations than seen during the first 8 h. Where the "equilibration period" for neurogenic effects on blood flow and oedema after microdialysis catheter insertion has previously been established as around one hour (11, 17, 18), this period for cytokine production is clearly longer and a much more complex issue. In the present protocol, the situation is not complicated by the use of topical local anaesthetic in the catheter membrane area. The amount of anaesthetic injected at the point of guide insertion was very small. Although effects on the pro-inflammatory cytokines in the dermis are still evident at 24 h after catheter insertion, it is likely that provoked or diseased tissue will demonstrate much higher levels. Some data exists in the literature on cytokine levels measured in the normal/uninvolved dermis (7, 19), though not with the same temporal resolution as in the present study. The more detailed cytokine profile from normal skin in the present study can be used as a "normal material" in the interpretation of profiles obtained from studies in diseased skin, which may well show other temporal or quantitative changes.

We view the invariable production of 3 cytokines, IL1b, IL6 and IL8, as a manifestation of the dermis inherent reactive capabilities; an "innate" response to trauma. The innate immune system is our first line of defence and, in the skin, is composed of many different elements designed to respond rapidly to pathogen invasion or cell and tissue damage (20, 21). It includes both cellular and molecular components.

The cellular component of the innate immune system is made up of both immune and non-immune cells, e.g. monocytes, keratinocytes, dendritic cells, mast cells, macrophages, endothelial cells and fibroblasts (22), on which receptors such as Toll-like receptors (TLR) (20) have been found extra- and intracellularly. While the innate immune system has been considered foremost a defence against invading pathogens, a role is postulated for the innate immune system in an increasing number of inflammatory conditions, e.g. psoriasis (23) and contact dermatitis (24). The insertion of the microdialysis catheter implies low-level trauma affecting cellular components and has relevance to previous studies of the effects of mechanical distortion on a tissue (25) and cytokine production in traumatized tissue (26), although the trauma has often been more pronounced or more prolonged in these studies. The molecular components that make up the innate pathways are constitutively expressed or can be produced locally and secreted by the cells present in the epidermis and dermis (22). Besides exogenous activation, endogenous molecules, so-called danger signals, released from cells and tissue that has been damaged, can also activate the innate immune system. It has been shown that uric acid, lipids from necrotic cells and heat shock proteins (27, 28), bind to TLRs, which in turn initiate the formation of a protein complex that assembles in the cytosol. This complex, called an inflammasome, activates caspase 1, which enables secretion of an active form of IL1b, IL18 and IL33 pro-inflammatory cytokines, from the cell (29). Evidence for inflammasome activation in keratinocytes and macrophages following ultraviolet B (UVB) radiation and in contact hypersensitivity has been reported recently (30, 31). We suggest that the production of cytokines by the microdialysis catheter insertion may
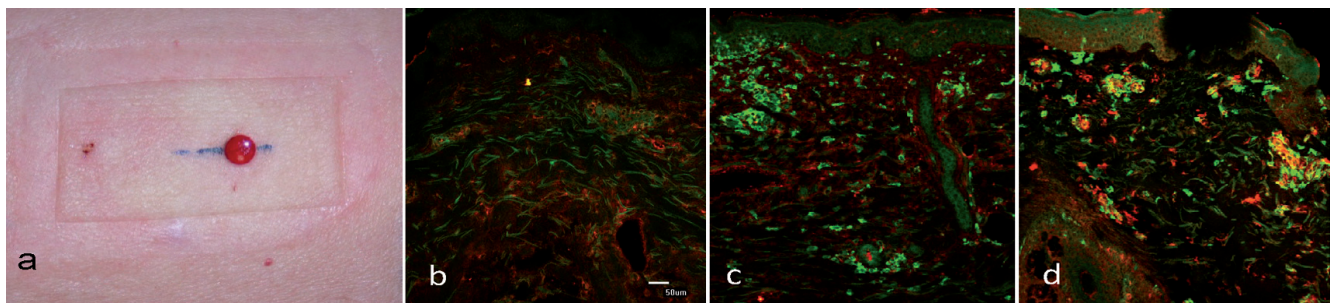
Fig. 4. (a–d) The area where the catheter membrane lay within the skin is marked by the blue line. A 4-mm diameter punch biopsy has been taken. (b–d) Microphotographs of cryostat sections representative for the 10 individuals. All sections are 20 × magnification and stained with anti-human leukocyte antigen HLA-DR-FITC (fluorescein isothiocyanate). Additionally, (b) is stained with anti-interleukin (IL)1b, (c) with anti-IL6 and (d) with anti-IL8. A green staining of the cell membrane is considered a positive finding for HLA-DR. Only bright-red particulate intracellular staining was considered a positive cytokine finding. Diffuse red staining of keratinocytes cell nuclei or the dead cells of the stratum corneum was judged to be artefactual. Of note is the absence of specific cytokine staining in the epidermis seen at the top of the pictures b–d.

be directly or indirectly the result of induction of the inflammasome.

In many inflammatory situations in the skin, cytokines produced in the epidermis will perfuse to the dermis, e.g. in the UVB reaction in which photons penetrate only as deep as the epidermis but still produce dermal (erythema and cellular infiltrates) reactions. In the present study, epidermal activation was not pronounced, as shown by the sparse findings of keratinocyte expression of HLA-DR. There were, however, prominent HLA-DR findings in the dermis, presumably identifying dermal macrophages and dendritic cells (32) and supporting the conclusion that the source of the cytokines in the microdialysates was predominately dermal. Which cells within the dermis are actually producing the cytokines in this study is as yet unclear, but several types of tissue cells are known to produce and store pro-inflammatory cytokines (33–35). The combination of microdialysis with end-point tissue biopsy gives complementary information in the experimental setting and is likely to be of further use in studies of diseased skin in which the number of cells in the dermis is greater.

For all cytokines, and especially those that were not detected, the question arises as to whether the membrane permitted passage of the molecule. This was examined *in vitro* by recovery experiments. The lowest recoveries were seen for TNFα, IL-10, IL-2 and IL-4 (5% and lower). Similar results for these molecules have been reported by others (19, 36). Thus some reservation in the interpretation of the low and negative microdialysis levels for these cytokines needs to be exercised. Better sensitivity might be gained if a trapping agent, such as heparin or bovine serum albumin, reactive with the different cytokines, were included in the perfusate. Also, it is possible to use other membranes, which may be needed for specific cytokines and the molecules generated in studies of inflammation (6, 37, 38).

While the innate immune system is designed primarily as a defence against bacteria and other microorganisms that can invade the skin barrier, it can also be triggered by tissue injury and destruction. It seems therefore reasonable to propose that the needle insertion causing sterile physical trauma studied in this paper is the equivalent of a "danger signal" to the dermis and that the production of cytokines seen demonstrates yet another level of the skin's innate immune response. Notwithstanding this innate reactivity, comparison of cytokine and chemokine production from diseased skin with that of normal skin of healthy individuals might give information about disease state or treatment response. A general conclusion of the present study is that microdialysis coupled with suspension array assay can be used *in vivo* in human skin to study quantitatively and qualitatively the chronology of cytokine production in the dermal intercellular space.

REFERENCES

1. Anderson C, Svensson C, Sjögren F, Andersson T, Wårdell K. Human in vivo microdialysis technique can be used to measure cytokines in contact reactions. Curr Probl Dermatol 1995; 23: 121–130.
2. Lönnroth P, Jansson PA, Smith U. A microdialysis method allowing characterization of intracellular water space in humans. Am J Physiol 1987; 253: E228–E231.
3. Petersen L, Kristensen J, Bülow J. Microdialysis of the interstitial water space in human skin in vivo: quantitative measurement of cutaneous glucose concentrations. J Invest Dermatol 1992; 99: 357–360.
4. Sjögren F, Svensson C, Anderson C. Technical prerequisites for in vivo microdialysis determination of interleukin-6 in human dermis. Br J Dermatol 2002; 146: 375–382.
5. Sjögren F. Dermal cell trafficking: from microscopy to microdialysis [medical]. Linköping: Unitryck Sweden; 2005.
6. Angst M, Clark J, Carvalho B, Tingle M, Schmelz M, Yeomans D. Cytokine profile in human skin in response to experimental inflammation, noxious stimulation, and administration of a COX-inhibitor: a microdialysis study. Pain 2008; 139: 15–27.
7. Averbeck B, Beilharz S, Bauer M, Gebhardt C, Hochleitner K, Kauer F, et al. In situ profiling and quantification of cytokines released during ultraviolet B induced inflammation by combining dermal microdialysis and protein microarrays. Exp Dermatol 2006; 15: 447–454.
8. Clough G, Jackson C, Lee J, Jamal S, Church M. What can microdialysis tell us about the temporal and spatial generation of cytokines in allergen induced responses in human skin in vivo. J Invest Dermatol 2007; 234: 443.
9. Anderson C. Cutaneous microdialysis: Is it worth the sweat? J Invest Dermatol 2006; 126: 1207–1209.
10. Anderson C, Andersson T, Andersson R. In-vivo microdialysis estimation of histamine in human skin. Skin Pharmacol 1992; 5: 177–183.
11. Groth L, Serup J. Cutaneous microdialysis in man: effects of needle insertion trauma and anaesthesia on skin perfusion, erythema and skin thickness. Acta Derm Venereol 1998; 78: 5–9.
12. Krogstad A, Jansson P, Gisslèn P, Lönnroth P. Microdialysis methodology for the measurement of dermal interstitial fluid in humans. Br J Dermatol 1996; 134: 1005–1012.
13. Andersson T, Wårdell K, Anderson C. Human in vivo cutaneous microdialysis: estimation of histamine release in cold urticaria. Acta Derm Venereol 1995; 75: 343–347.
14. Fulwyler M, McHugh T. Flow microsphere immunoassay for the quantitative and simultaneous detection of multiple soluble analytes. Methods Cell Biol 1990; 33: 613–629.
15. Andersson J, Abrams J, Björk L, Funa M, Litton M, Ågren K, et al. Concomitant in vivo production of 19 different cytokines in human tonsils. Immunol 1994; 83: 16–24.
16. Willis C, Stephens C, Wilkinson J. Selective expression of immune-associated surface antigens by deratinocytes in irritant contact dermatitis. J Invest Dermatol 1991; 96: 505–511.
17. Anderson C, Andersson T, Wårdell K. Changes in skin circulation after insertion of a microdialysis probe visualized by laser Doppler perfusion imaging. J Invest Dermatol 1994; 102: 807–811.

18. Petersen L. Measurement of histamine release in intact human skin by microdialysis. Clinical and experimental findings. [DSC]: Copenhagen, Denmark: The Danish Medical Association Publishing Co.; 1998.
19. Clough G. Microdialysis of large molecules. AAPS J 2005; 7: E686–E692.
20. Janeway C, Medzhitov R. Innate immune recognition. Ann Rev Immunol 2002; 20: 197–216.
21. McInturff J, Modlin R, Kim J. The role of toll-like receptors in the pathogenesis and treatment of dermatological disease. J Invest Dermatol 2004; 125: 1–8.
22. Clark R, Kupper T. Old meets new: the interaction between innate and adaptive immunity. J Invest Dermatol 2005; 125: 629–637.
23. Bos J, de Rie M, Teunissen M, Piskin G. Psoriasis: dysregulation of innate immunity. Br J Dermatol 2005; 152: 1098–1107.
24. Bonneville M, Chavagnac C, Vocanson M, Rozieres A, Benetiere J, Pernet I, et al. Skin contact irritation conditions the development and severity of allergic contact dermatitis. J Invest Dermatol 2007; 127: 1430–1435.
25. Ryan T. Biochemical consequences of mechanical forces generated by distention and distortion. J Am Acad Dermatol 1989; 21: 115–130.
26. Perl M, Gebhard F, Knöferl M, Bachem M, Gross H, Kinzl L, et al. The pattern of reformed cytokines in tissues frequently affected by blunt trauma. Shock 2003; 19: 299–304.
27. Shi Y, Evans J, Rock K. Molecular identification of a danger signal that alerts the immune system to dying cells. Nature 2003; 425: 516–521.
28. Matzinger P. The danger model: a renewed sense of self. Science 2002; 296: 301–305.
29. Martinon F, Burns K, Tschopp J. The inflammosome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-B. Mol Cell 2002; 10: 417–426.
30. Feldmeyer L, Keller M, Niklaus G, Hohl D, Werner S, Beer H. The inflammasome mediates UVB induced activation and secretion of interleukin 1b by keratinocytes. Curr Biol 2007; 17: 1140–1145.
31. Watanabe H, Gaide O, Pétrilli V, Martinon F, Contassat E, Roques S, et al. Activation of the IL1b processing inflammasome is involved in contact hypersensitivity. J Invest Dermatol 2007; 127: 1956–1963.
32. Meunier L, Gonzalez-Ramos A, Cooper K. Heterogeneous populations of classII MHC+ cells in human dermal cell suspensions. J Immunol 1993; 151: 4067–4080.
33. Debets R, Hegmans J, Deleuran M, Hooft S, Brenner R, Prens E. Expression of cytokines and their receptors by psoriatic fibroblasts I. Altered IL6 synthesis. Cytokine 1996; 8: 70–79.
34. Gordon J, Galli S. Mast cells as a source of both preformed and immunologically inducible TNF-alpha/cachectin. Nature 1990; 346: 274–276.
35. Utgaard J, Jahnsen F, Bakka A, Brandtzaeg P, Haraldsen G. Rapid secretion of prestored interleukin 8 from Weibel-Palade bodies of microvascular endothelial cells. J Exp Med 1998; 188: 1751–1756.
36. Ao X, Stenken J. No delayed temporal response to sample concentration changes during enhanced microdialysis sampling using cyclodextrins and antibody-immobilized microspheres. Analyst 2006; 131: 61–67.
37. Ao X, Sellati T, Stenken J. Enhanced microdialysis relative recovery of inflammatory cytokines using antibody coated microspheres analysed by flow cytometry. Anal Chem 2004; 76: 3777–3784.
38. de Jong C, Lutter R, Verberk M, Kezic S. Differential cytokine expression in skin after single and repeated irritation by sodium lauryl sulphate. Exp Dermatol 2007; 16: 1032–1040.