MACROPHAGE INFLUX AND PHENOTYPE DURING NORMOTROPHIC AND HYPERTROPHIC SCAR FORMATION IN HUMANS
ABSTRACT

Tissue macrophages can display different functional phenotypes. M1 macrophages have an increased expression of CD40 and exhibit antimicrobial properties releasing of inflammatory mediators including tumor necrosis factor (TNF)-α and interleukin (IL)-12. M2 macrophages produce transforming growth factor (TGF)-β and IL-10 and express both mannose receptor (CD206) and haemoglobin scavenger receptor (CD163). The M2 phenotype has been linked to fibrosis. The aim of this study was to determine the macrophage influx and phenotype associated with normotrophic and hypertrophic scar formation in time, up to 52 weeks after surgery. Human presternal wound healing after cardiothoracic surgery through a sternotomy incision was investigated in a standardized manner. Skin biopsies were collected at consecutive time points, i.e., during surgery and 2, 4, 6, 12, and 52 weeks postoperatively. The number and distribution of CD45+ CD68+, CD40+, CD163+ and CD206+ cells were evaluated. The expression levels of type I collagen, type III collagen, TNF-α, IL-12, TGF-β1 and IL-10 were measured by real-time reverse transcription-polymerase chain reaction. Hypertrophic scars had an increased collagen type III production 52 weeks after surgery. In the hypertrophic group, an increased number of CD68+ cells was seen 4 and 6 weeks after injury, and both CD40+ and CD163+ were present in higher number after 6 weeks, compared with the normotrophic group. When comparing the hypertrophic group and normotrophic group at these time points, no significant differences in expression levels of TNF-α, and IL-12, TGF-β1 and IL-10 were found. In conclusion, scars developing toward hypertrophic scars display an increased number of macrophages 4 and 6 weeks after injury. These macrophages have an increased expression of CD163 and CD40. More knowledge of the role of macrophages in human cutaneous wound healing is necessary, in order to establish their functional phenotype in vivo in the different phases of wound repair and in different pathological conditions.

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INTRODUCTION

The formation of scars is a natural part of cutaneous wound healing in humans. In many individuals, however, the wound healing process can lead to the production of overabundant extracellular matrix (ECM), resulting in hypertrophic scars\(^1\). These scars are raised, red, inflexible and responsible for serious functional and cosmetic problems.

The exact underlying mechanism of hypertrophic scar formation is unknown. It is becoming increasingly clear, however, that the inflammatory phase of wound healing, besides being essential for preventing infection, plays an important role in fibrogenesis at sites of tissue repair\(^1\). Macrophages are considered key players in wound healing, as they serve as a source for cytokines and chemokines essential for orchestrating the wound healing process and ECM production. Since the role of macrophage phenotype has been highlighted in fibrosis in several studies, while other studies underline the importance of macrophages in wound healing, the interest in macrophage function in wound repair is rapidly increasing.

Tissue macrophages, arisen from monocytes, can display different functional phenotypes. Mediators that stimulate macrophages to differentiate into different functional phenotypes are typically derived from activated T helper cells\(^2\). The currently most widely used classification scheme for the activation of macrophages defines classically activated as M1, and the group of non-classically activated (or alternatively activated) as M2\(^3\). In the classical activation of macrophages, interferon (IFN)-\(\gamma\) converts resting macrophages into so-called M1 macrophages that have an increased expression of CD40 and exhibit antimicrobial properties by release of inflammatory mediators including tumor necrosis factor (TNF)-\(\alpha\), nitric oxide (NO) and interleukin (IL)-6 and IL-12\(^4,5\). On the other hand, macrophages activated by IL-4 and IL-13 develop into M2 macrophages\(^5\), which produce TGF-\(\beta\) and IL-10 and express both mannose receptor (CD206) and haemoglobin scavenger receptor (CD163) in humans\(^6-8\). T helper 2 (Th2) responses, associated with a high production of IL-4 and IL-13, are essential for the defense against parasitic infections, but also contribute to allergy, asthma and fibrosis\(^4,9,10\). And more specifically, a relatively high expression of cytokines such as TGF-\(\beta\) and IL-4, associated with an M2 and Th2 phenotype, respectively, is associated with hypertrophic scar formation\(^11-15\).

The above-mentioned macrophage phenotypes are actually extremes of a continuum of macrophage function and have not been determined in pure form in vivo\(^6,16\). The observed phenotype may vary depending on the complex mixture of cytokines and other ligands in the microenvironment, and wound macrophages may even exhibit a complex progression of phenotypes\(^17,18\). Furthermore, discrepancies are found between mouse and human models of alternative activation of macrophages\(^2\). The current literature lacks in vivo characterization of the receptor expression and cytokine production kinetics by macrophages during cutaneous wound healing in humans\(^19\). Thus, whether the proposed phenotypes actually represent macrophage populations in the human host, and how these phenotypes are represented in the human wound during normotrophic and hypertrophic scar formation is currently unknown.
Despite new insights in molecular and cellular abnormalities associated with hypertrophic scar formation, a high-quality therapy to prevent hypertrophic scar formation is still lacking. This may be a consequence of poor extrapolation of observations in \textit{in vitro} cell systems and animal models to the wound in a patient. In studies investigating human wound healing, and especially the inflammatory response, interactions in wound and scar tissue, both via cell-cell contact and via secreted products, are spatiotemporally controlled and difficult to mimic in \textit{in vitro} cell systems. Hence, studying cell (dys)function in the complex microenvironment of the human wound remains a prerequisite.

The aim of this study was to determine the macrophage influx and phenotype associated with normotrophic and hypertrophic scar formation in time. Human presternal wound healing after cardiothoracic surgery through a sternotomy incision was investigated in a standardized manner. Skin/scar biopsies were collected at six time points; during surgery (control sample), 2, 4, 6, 12, and 52 weeks postoperatively. The number and distribution of CD45\(^{+}\), CD68\(^{+}\), CD40\(^{+}\), CD163\(^{+}\) and CD206\(^{+}\) cells were evaluated. Expression levels of the genes associated with classically- and alternatively-activated macrophages were investigated.

**MATERIALS AND METHODS**

**Patients and tissue samples**

Human presternal wound healing was investigated in a standardized manner as previously described\(^20\). Patients older than 18 years undergoing cardiothoracic surgery through a median sternotomy incision were assessed for eligibility of participation in the study. Ultimately, a total of 42 patients were included. Approval of the medical ethics committee of the University Medical Center Groningen was obtained and all the participants gave written consent.

All operations were performed at the Department of Cardiac Surgery of the University Medical Center Groningen. During surgery, from all patients a skin tissue sample of the caudal part of the sternotomy incision was collected (control sample). Evaluations took place under standardized conditions 2, 4, 6, 12 and 52 weeks after surgery, and were performed by the same observer. During all evaluations the same presternal scar was evaluated at 8 cm from the cranial and the caudal border and scored as normotrophic or hypertrophic. Hypertrophic was defined as raised above skin level (> 1 mm) while remaining within the borders of the original lesion\(^21\). Normotrophic was defined as not raised above skin level. The height and width of the scar were measured 12 and 52 weeks postoperatively using a slide caliper and a 7.5 MHz ultrasound probe (SSD-680 EX/STD, Aloka Co., Ltd., Tokyo, Japan). These data have been described in detail previously\(^20\). Patients were classified as hypertrophic or normotrophic when the presternal scar was hypertrophic or normotrophic respectively 12 and 52 weeks postoperatively.

During the evaluations, a 3 mm punch biopsy was collected from the caudal segment of the scar after local anesthesia with 5 mL of lidocaine hydrochloride (10 mg/mL) combined with epinephrine (0.01 mg/mL), consecutively at 3, 4, 5, 6, and 7
cm from the caudal confine of the scar. After collection, all samples were snap frozen on liquid nitrogen and stored at -80°C until further processing. For real-time reverse transcription-polymerase chain reaction (RT-PCR), to quantify gene expression, and immunohistochemistry to localize protein expression, the same tissue specimens were used. After the final evaluation of the last participant, a control group consisting of patients who had developed a complete normotrophic presternal scar in both the cranial and caudal segment 12 and 52 weeks postoperatively was matched to the hypertrophic group with respect to age, sex and body-mass index, and was defined as the normotrophic group.

Real-time RT-PCR
Total RNA was isolated from all samples and prepared for real time RT-PCR as previously described. Exons overlapping primers and minor groove binder probes for real-time PCR were purchased as Assay-on-Demand from Applied Biosystems (Nieuwekerk a/d IJssel, The Netherlands): GAPDH (assay ID Hs99999905_m1), COL1A2 (Hs00164099_m1), COL3A1 (Hs00164103_m1), TGF-β1 (Hs00998133_m1), IL10 (Hs00961622_m1), IL12A (Hs01073447_m1), IL12B (Hs01011518_m1), and TNF (Hs00174128_m1). TaqMan real-time PCR was performed in an ABI PRISM 7900HT Sequence Detector (Applied Biosystems). Duplicate real-time PCR analyses were executed for each sample, and the threshold cycle (Ct) values obtained were averaged. Gene expression was normalized to the expression of the housekeeping gene GAPDH. Relative mRNA expression is presented as the percentage relative to the mRNA expression in the control sample (t=0) +/- SD.

Immunohistochemistry and immunofluorescence
The frozen biopsy samples were oriented on edge with the line of visible epidermal scar tissue perpendicular to the plane of section and processed for 5 μm cryostat sections. After fixation in acetone, sections were incubated with primary antibodies for 60 minutes. All primary antibodies and concentrations used are summarized in Table 1. For immunofluorescence, the appropriate Alexa Fluor® 555 nm-conjugated secondary antibodies (Invitrogen, Paisley, UK) were applied. Immunohistochemical detection was performed using two-stage alkaline phosphatase (AP) EnVision (Dako, Glostrup, Denmark) according to the protocol of the manufacturer. The sections were counterstained with Mayer haematoxylin and mounted in Glycergel (Dako). As controls, sections were stained as described using the appropriate isotype-matched negative control antibodies, and were always devoid of color development.

Positive cells were scored quantitatively by computerized scoring of the percentage of positive staining in five randomly chosen high-powered fields (HPF = visual field at ×100 magnification). The final score for each biopsy is the average score of the five HPF.

Statistical analysis
Differences between groups regarding gene expression (relative to the expression of GAPDH) were tested using the Wilcoxon (for paired data) and the Mann-Whitney (for
CHAPTER 7

unpaired data) tests. To adjust for multiple testing when testing for differences in gene expression patterns between groups, the Benjamini-Hochberg method\textsuperscript{23} was applied to control the false discovery rate. Only those results whose $P$-values yielded a false discovery rate below 0.10 and a $P$-value below 0.05 were considered to be significant. The same procedure was applied to relative gene expression data, which were obtained by subtracting the gene expression at 0 weeks (control sample) from the gene expression at 2, 4, 6, 12 and 52 weeks, to remove the inter-patient variation\textsuperscript{24}. Data analysis was performed using S-plus statistical software (Insightful Corp., Seattle).

RESULTS

Hypertrophic scar formation in the study group
A total of 76 patients were included, of which 42 were able to complete the follow up of 52 weeks. Other patients discontinued participation due to their medical condition in the period between two and four weeks after surgery. One patient underwent surgery for cardiac valve replacement, two for a heart defect and 39 for coronary artery bypass surgery (see Table 2 for patient characteristics).

The caudal segment of the presternal scars was most susceptible to hypertrophic scar formation. In 5 patients this scar segment was hypertrophic at 12 and 52 weeks postoperatively. Of the 27 patients who had developed normotrophic scars in both the cranial and the caudal segment 12 and 52 weeks postoperatively, six were matched with the patients in the hypertrophic group with respect to age, sex and body-mass index, and considered the normotrophic group (Table 2).

Table 1. Antibodies used in immunohistochemistry.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Isotype</th>
<th>Dilution*</th>
<th>Company</th>
<th>Code number</th>
</tr>
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<tr>
<td>Mouse anti-CD45 (MEM-28)</td>
<td>IgG1</td>
<td>1/50</td>
<td>IQ Products, Groningen, the Netherlands</td>
<td>IQP-549P</td>
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<td>Mouse anti-CD68 (EBM11)</td>
<td>IgG1</td>
<td>1/100</td>
<td>Dako, Glostrup, Denmark</td>
<td>M0718</td>
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<td>Mouse anti-CD40 (LOB7/6)</td>
<td>IgG2a</td>
<td>1/100</td>
<td>AbD Serotec, Oxford, UK</td>
<td>MCA1590</td>
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<tr>
<td>Mouse anti-CD163 (EDHu-1)</td>
<td>IgG1</td>
<td>1/100</td>
<td>AbD Serotec, Düsseldorf, Germany</td>
<td>MCA1853</td>
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<td>Mouse anti-CD206 (19.2)</td>
<td>IgG1</td>
<td>1/100</td>
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<td>555953</td>
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<td>Goat anti-mouse Alexa Fluor 555</td>
<td>IgG</td>
<td>1/800</td>
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<tr>
<td>Streptavidin, Alexa Fluor - 555 Conjugate</td>
<td>1/800</td>
<td>Invitrogen, Paisley, UK</td>
<td>S-32355</td>
<td></td>
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</tbody>
</table>

* Diluted in 1% BSA/PBS.
Table 2. Patient characteristics of the study group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study group</th>
<th>Hypertrophic group</th>
<th>Normotrophic group</th>
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<td>5/0</td>
<td>5/1</td>
</tr>
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<td>Age, mean (range), y</td>
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<td>49 (40-55)</td>
<td>56 (46-63)</td>
</tr>
<tr>
<td>Weight, mean (range), kg</td>
<td>83.6 (56-124)</td>
<td>93 (56-108)</td>
<td>82 (58-124)</td>
</tr>
<tr>
<td>Length, mean, m</td>
<td>1.77</td>
<td>1.82</td>
<td>1.82</td>
</tr>
<tr>
<td>BMI, mean</td>
<td>26.6</td>
<td>27.9</td>
<td>24.8</td>
</tr>
<tr>
<td>Smoking, No. (%)</td>
<td>12 (27)</td>
<td>2 (40)</td>
<td>3 (50)</td>
</tr>
</tbody>
</table>

Collagen mRNA expression was upregulated in hypertrophic scars up to 12 months postoperative

To study whether the hypertrophic scars were associated with a higher collagen synthesis, we examined the mRNA expression of both type I and type III collagen. The relative mRNA levels in both groups are summarized in Figure 1. The mRNA expression of type I and type III collagen significantly increased 12 weeks postoperatively in both the hypertrophic and the normotrophic group. The mean (SD) type I collagen expression in the normotrophic and hypertrophic group increased by 862% (171%) and 828% (720%), respectively (P < 0.001), while the type III collagen expression increased by 1393% (416%) and 1180% (892%), respectively (P < 0.001). In the hypertrophic group, however, the mRNA levels of type III collagen continued to increase between 12 and 52 weeks postoperatively, while in the normotrophic group levels declined. This resulted in a significantly higher mean (SD) type III collagen expression in the hypertrophic group versus the normotrophic group (1732% [443%] versus 527% [402%]; P < 0.01) 52 weeks postoperatively.

Figure 1. Time course of mRNA expression of Type I and Type III collagen in the hypertrophic and normotrophic group. Relative mRNA expression is presented as the percentage relative to the mRNA expression in the control sample (t=0) ± SD. *p < 0.05.
Hypertrophic scar formation was preceded by a higher influx of CD163+ and CD40+ macrophages

Quantification of CD45+ cells is shown in Figure 2 and revealed that there was a higher number of inflammatory cells present in the skin during hypertrophic scar formation compared with normotrophic scar formation (P < 0.001). To determine whether this represented a difference in the number of macrophages, we scored the cells positive for CD68, a pan-macrophage marker. The scoring of CD68-stained sections is shown in Figure 2. The number of CD68-positive cells in the skin was increased 2 weeks postoperatively compared with the control sample (collected during surgery) in both groups (P = 0.02). In the hypertrophic group, the percentage of CD68-positive cells continued to increase thereafter, yielding a significantly higher mean CD68 score in the hypertrophic group 6 weeks postoperatively compared with the normotrophic group (P = 0.03). The number of macrophages was increased both in the perivascular regions and between collagen bundles (Figure 3).

To explore the phenotype of the CD68-positive population in more detail, we examined and quantified the expression of CD40, CD163 and CD206 (Figure 2). In the normotrophic group, the expression of CD40, a cell surface marker associated with classical activation of macrophages (M1), and CD163 (haemoglobin scavenger receptor) associated with alternative activation (M2), followed the trend of the expression of CD68. In the normotrophic group, both CD40 and CD163 were expressed at peak level 2 weeks postoperatively, before returning to baseline level.
thereafter. In the samples in the hypertrophic group, in contrary, there was a peak expression of CD163 after 4 and 6 weeks, and of CD40 after 6 weeks postoperatively, which resulted in a significantly higher expression of CD40 ($P < 0.001$) and CD163 ($P < 0.001$) in the hypertrophic group 6 weeks postoperatively. CD40 was mainly expressed perivascularly (Figure 4). CD163 expression was upregulated among macrophages throughout the tissue samples (Figure 5). When comparing the CD206 expression in both groups, it followed a similar trend, with generally the highest expression between 2 and 6 weeks postoperatively (Figure 2). There was no significant difference in expression between both groups.

M1- and M2-associated cytokines in hypertrophic versus normotrophic scar formation

To further analyze the macrophage phenotype in the skin samples, we analyzed the mRNA expression of cytokines typically (although not exclusively) expressed by M1 macrophages (TNF-α, IL-12A and IL-12B) and M2 macrophages (IL-10, and TGF-β1). The time course of the mRNA expression of these genes in the skin samples of the normotrophic and hypertrophic group is shown in Figure 6. In the control skin samples, there was no significant difference in mRNA expression of the above-mentioned genes between the hypertrophic and normotrophic group.

From the M1-associated cytokines, only TNF-α was expressed differently between both groups. The mRNA expression of TNF-α was significantly higher in de normotrophic group ($P = 0.001$) mainly due to the increased expression between 6 and 52 weeks postoperatively, while in de hypertrophic group levels stayed on or below baseline level throughout the follow-up period. Regarding IL-12A and IL-12B, in both groups a similar pattern in mRNA expression was seen, and no evidence of a difference in expression of these cytokines between the groups was found.
Regarding the M2-associated cytokines, in both groups IL-10 expression was significantly increased after 2 weeks compared with the control sample ($P < 0.001$), after which it started to decline to return to baseline level 52 weeks postoperatively. There was no significantly different IL-10 expression in the hypertrophic compared with the normotrophic group. TGF-β1 mRNA was expressed at higher levels in the hypertrophic group 2 weeks postoperatively ($P = 0.007$), after which the mean expression was continuously higher in the hypertrophic group, although, due to high variation, not statistically significantly different from the normotrophic group.

<table>
<thead>
<tr>
<th>Time after surgery</th>
<th>0 weeks</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>6 weeks</th>
<th>12 weeks</th>
<th>52 weeks</th>
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<tr>
<td>Normotrophic</td>
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</tbody>
</table>

Figure 4. CD40-stained sections of hypertrophic and normotrophic presternal scars at all time points.

<table>
<thead>
<tr>
<th>Time after surgery</th>
<th>0 weeks</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>6 weeks</th>
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Figure 5. CD163-stained sections of hypertrophic and normotrophic presternal scars at all time points.
towards hypertrophic scars, that have an increased collagen type up to 52 weeks after surgery. For the associated with both normotrophic and hypertrophic scar formation in humans in time, relative to the mRNAs of hypertrophic and normotrophic group.

Figure 6. Time course of mRNA expression of M1- and M2-associated cytokines in the hypertrophic and normotrophic group. Relative mRNA expression is presented as the percentage relative to the mRNA expression in the control sample (t=0) ± SD. *P < 0.05.

DISCUSSION

The aim of this study was to determine the cellular influx and macrophage phenotype associated with both normotrophic and hypertrophic scar formation in humans in time, up to 52 weeks after surgery. For the first time we established that scars developing towards hypertrophic scars, that have an increased collagen type III production 52 weeks after surgery, display an increased macrophage influx 4 and 6 weeks after injury. These macrophages express an increased level of CD163 and CD40. When comparing the hypertrophic group and normotrophic group at these specific time points, no significant differences in expression levels of typical M1- or M2-associated cytokines were found. Therefore, an association between hypertrophic scar formation and a macrophage phenotype that fits the current dogmas on macrophage activation and differentiation in vitro could not be established in vivo.

Numerous studies have demonstrated that cells belonging to the monocyte-macrophage lineage represent a heterogeneous group of cells with distinct functional phenotypes, capable of secreting various profibrotic cytokines. They are highly sensitive to microenvironment-controlled signals, which determine their differentiation into a particular functional phenotype. Two extremes of a continuum of possible differentiation routes have been identified, but their actual presence in vivo is subject of discussion. The M1 macrophages exhibit antimicrobial properties by release of inflammatory mediators like TNF-α, NO, IL-6 and IL-12, while the M2 macrophages release TGF-β1 and IL-10, express both mannose receptor (CD206) and haemoglobin scavenger receptor (CD163) and are said to suppress the inflammatory response and promote angiogenesis, tissue remodeling and repair. Ultimately, it seems that not the
inflammatory response *per se*, but the actual phenotype of this inflammatory response is decisive for the scar outcome.

Alternatively-activated macrophages have been linked to fibrosis\(^1\), where they are thought to play a major role in the early stages of disease. Furthermore, modification of the early inflammatory response with glucocorticoids, which are known to stimulate the alternative activation of macrophages\(^2\), increase scar dimensions during the later stages of wound healing in patients that develop hypertrophic scars\(^20\). In light of these findings, we hypothesized that during wound healing associated with excessive scarring more macrophages are attracted to the wound bed, and that these attracted macrophages will display a more alternatively-activated M2 phenotype. Our data show that in scar tissue samples developing into hypertrophic scars an increased number of macrophages is present at the stage before the hypertrophic scar becomes present, i.e., 4 and 6 weeks postoperatively. These macrophages have an increased expression of CD163 and CD40, which are known to be markers for M1 and M2 macrophages, respectively. The expression of mannose receptor (CD206), another M2 marker\(^6\), was similar in both groups, with a slightly higher expression level 2, 4 and 6 weeks postoperatively compared with the control samples. The expression levels of typical M1- or M2-associated cytokines were not significantly higher in the hypertrophic group at the time points at which we observed a higher number of CD163\(^+\) and CD40\(^+\) macrophages. Therefore, an association between hypertrophic scar formation and a macrophage phenotype that fits the current dogmas on macrophage activation and differentiation could not be established in these human tissue samples. It should however be noted that TGF-β1 mRNA was expressed at higher level in the hypertrophic group 2 weeks postoperatively, which could be an effect attributed to the early inflammatory phase.

For the wound healing process to occur uncomplicated, macrophages are crucial mainly during the early inflammatory response (1-4 days after injury) and during granulation tissue formation (4-10 days after injury)\(^25\). Depletion of macrophages after 10 days does not impair wound healing. We cannot attribute differences in scar outcome to a difference in macrophage function early in wound healing as we did not analyze samples collected earlier than two weeks after injury. Whether the presence of the higher number of macrophages in the hypertrophic group 4 and 6 weeks after injury has been influenced by differences in the phenotype of the early inflammatory response remains a question that needs to be addressed in future studies, but seems likely\(^14,20\).

For the first time, we investigated the time course of the influx of macrophages and the expression of different markers and cytokines associated with macrophage polarization and differentiation during the process of hypertrophic scar formation in the human wound. The investigation of sequentially collected scar tissue samples from the same scars in the same patients during the scar formation and remodeling process has provided valuable information regarding human hypertrophic scar formation without inter-patient variability. Nonetheless, our findings also have limitations. One of the reasons why we were not able to establish a macrophage phenotype and detect differences in expression of macrophage-associated cytokines between both groups
may lie in the fact that we were not able to collect tissue samples between surgery and 2 weeks after surgery, during which most inflammatory processes take place, and which is believed to be the phase were the stage is set for the wound healing and scar formation process. Furthermore, we analyzed mRNA expression of cytokines using total RNA from the 3 mm specimen biopsies, by which we possibly missed local differences in gene expression, as compartmentalization of the different processes and cells is likely. As can be seen in immunohistochemical analysis, inflammatory cells are often clustered together, and a difference in number of inflammatory cells and cytokine levels may be influenced by the fact that an area of the specimen is analyzed that is not representative for the whole tissue sample. In the future, laser microdissection followed by genomic and proteomic analysis of macrophages from human wound biopsies might be helpful in determining the expression of cytokines produced by macrophages or macrophage subsets more specifically, and subsequently to determine the macrophage phenotype in vivo. This will hopefully provide us with a better understanding of the actual macrophage phenotype and function during normal human wound healing and wound healing associated with hypertrophic scar formation.

In conclusion, we have shown that scars developing towards hypertrophic scars display an increased number of macrophages 4 and 6 weeks after injury. These macrophages have an increased expression of CD163 and CD40. Intervention in macrophage function during hypertrophic scar formation may eventually result in a better outcome. Before intervention in these complex processes is justified, more knowledge of the role of macrophages in human cutaneous would healing is necessary, in order to establish their functional phenotype in vivo in the different phases of wound repair and in different pathological conditions.

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