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Summary

Due to the increasing ages of the Western populations and to the increasing technical possibilities, biomaterials are increasingly used in the clinic. Examples of biomaterials are hip and breast prostheses, artificial heart valves, wound dressings, nerve guides or drug delivery systems. However, unwanted foreign body reactions still occur, leading to implant failure, infections and the necessity for re-operations. Biocompatibility, defined as the ability of a biomaterial to perform with an appropriate host response in a specific application [1,2], could possibly be improved by interfering in the foreign body reaction, e.g., by inhibiting macrophage (MØ) attraction and activation, or by inhibiting giant cell formation and activation. But first, the cascade of the foreign body reaction should be understood.

The subjects described in this thesis concern the fundamental understanding and the modulation of the foreign body reaction to the biodegradable biomaterial hexamethylenediisocyanate crosslinked dermal sheep collagen (HDSC) in AO rats at the level of the T cell and and its MØ-activating cytokine interferon-γ (IFN-γ) (chapters 2 to 4). Furthermore, the role of IFN-γ was studied in IFN-γ-receptor knock-out mice (chapter 5). Finally, the foreign body reaction to HDSC in rats and mice was compared and attempts were made to understand and to enhance the reaction in mice (chapter 6 and 7).

Although in biomaterial-research, the involvement of T cells is often neglected, it was demonstrated in this study that T cells play an important role in the foreign body reaction to HDSC in rats (chapter 2). The lack of T cells in athymic nude rats delayed the foreign body reaction to HDSC for approximately 3 weeks. In control rats, some T cells (less than 1%) were detected in or near the HDSC disk. It could be questioned whether these T cells have a specificity against the biomaterial. Probably, the T cells involved in the foreign body reaction have a more regulatory function, for example, production of IFN-γ to induce activation of MØ.

In chapters 3 and 4, it was studied whether IFN-γ is involved in the foreign body reaction to HDSC in rats. Local application of neutralizing antibodies against IFN-γ clearly delayed the foreign body reaction. Cellular ingrowth, expression of MHC class II, which is involved in antigen presentation, and degradation of HDSC were inhibited, showing that modulation of the foreign body reaction is possible. In contrast to local impregnation, systemic application of anti-IFN-γ only inhibited the expression of MHC class II. These discrepancies are not fully understood. However, in clinical situations, it is more preferable to treat patients locally due to the higher risk of negative side effects and due to financial aspects (higher dosages).

To investigate the role of IFN-γ in further detail, knock-out mice (129 SVEV strain) in which the gene for the IFN-γ-receptor was disrupted, might provide an elegant model (chapter 5). Unexpectedly, no differences in the foreign body reaction to HDSC between the knock-out and wild-type mice were found, leaving the question unsolved whether IFN-γ is an important factor in the foreign body reaction or not. Obviously, phagocytosis of the HDSC bundles by giant cells was delayed up to week 9 in both knock-out and wild-type mice, in contrast to rats which phagocytose the HDSC bundles within 2 weeks.

In biomaterial research both mice and rats are used as *in vivo* models to test biomaterial's biocompatibility and functionality. In chapter 6, it was shown that the reaction to HDSC significantly differed between rat and mouse. The foreign body reaction in mice was rather variable. But overall in mice, giant cell formation and ingrowth was slower, while stroma formation was stronger in comparison to rats. Moreover, in mice, phagocytosis of the HDSC bundles hardly occurred, MHC class II expression was minimal, and HDSC calcified more often. Although all mice strains tested (i.e., 129 SVEV, BALB/c, and C57BL/6) reacted comparable, differences were observed between the rat strains tested (i.e., AO, BN, F344, LEW, and PVG). Plasma cells infiltrated the HDSC disc in PVG rats while T cells infiltrated the disc in LEW and F344 rats. In contrast, AO rats, which were used for many previous studies at our laboratory [3-5] had no infiltration of plasma cells and hardly infiltration of T cells. It may be of interest to study whether other types of biomaterials induce similar variation in foreign body reaction.

These results can have a great impact on future biomaterial studies. Although we still speculate about the human situation, results could be too positive due to the minor foreign body reaction, when materials are only tested in mice. However, because the mouse seems to be more sensitive for calcification, this animal might prove to be a sensitive model for calcification studies. For modulation studies in which biomaterials are pre-treated with (antibodies to) cytokines or with other factors like extracellular matrix proteins, the rat might be more preferable because of the strong foreign body reaction. But one should be critical in choosing a specific rat strain.

It was hypothesized that the minimal foreign body reaction in mice might be due to an impairment in extracellular degradation of the HDSC bundles by proteolytic enzyms. In chapter 7, HDSC was pre-degraded *in vitro* in order to present smaller particles which should be easier to internalize by MØ and giant cells. However, the presence of pre-degraded HDSC hardly enhanced the giant cells activity, although the expression of the matrix metalloproteinase-9 was enhanced. In additional experiments, HDSC was impregnated with the pro-inflammatory cytokine tumor necrosis factor-α or with IFN-γ before implantation. However, no enhancement of giant cell activity was observed with either treatment.

This thesis demonstrates that T cells and IFN-γ are important factors in the foreign body reaction to HDSC in rats. Moreover, modulation of the foreign body reaction, in the sense of inhibiting MØ and giant cell activity, by local administration of neutralizing anti-IFN-γ antibodies is possible. An important finding was that the foreign body reaction to HDSC clearly differs between rats and mice. In mice, less giant cells are formed which were hardly activated according to phagocytosis and MHC class II expression. Angiogenesis and stroma formation were not inhibited despite the minor giant cell activity. The factors leading to these differences in rats and mice are not fully inderstood. But it can be hypothesized that MØ and giant cells are pivotal cell types in the foreign body reaction. Further studies might unravel the function of these cell types in these animal models with respect to biocompatibility and tissue engineering. On the other hand, repair of autologous tissue including angiogenesis should be stimulated. Future research will result in therapeutic strategies to modulate the foreign body reaction and improve biocompatibility.

References

1. Williams DF, *Progress in Biomedical Engineering 4*. Definitions in Biomaterials, ed. D.F. Williams. Vol. 4. 1987, Amsterdam: Elsevier Science Publishers B.V. 72.
2. Williams DF, *The Williams Dictionary of Biomaterials.* 1999, Liverpool: Liverpool Univeristy Press.
3. Wachem PB van, Luyn MJA van, Olde Damink L, Feijen J, Nieuwenhuis P, Tissue interactions with dermal sheep collagen implants: a transmission electron microscopical evaluation. Cells Mater 1991; 1:251-263.
4. Wachem PB van, Luyn MJA van, Olde Damink LHH, Dijkstra PJ, Feijen J,