Autologous Nucleus Pulposus Attracts Monocytes In Vivo In A Pig Model. Implications For The Pathophysiology Of Sciatica.

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Abstract

Previous experimental studies have indicated that that an autoimmune reaction towards herniated nucleus pulposus may constitute a pathogenetic component in the pathophysiology of sciatica. If an autoimmune reaction would be present, one would expect that exposure of nucleus pulposus to the host would induce an up regulation of antigen-presenting cells, such as monocytes. Autologous nucleus pulposus was placed in perforated titanium chambers, which were placed subcutaneously in pigs, together with an equal amount of empty chambers for control. After 7 days, the chambers were harvested and the number of monocytes in the chambers was determined using flow cytometry. The proportion of monocytes was significantly higher in the nucleus pulposus filled chambers than in the empty chambers. In conclusion, nucleus pulposus was shown to attract monocytes, which may suggest that monocytes might play a role in the pathophysiology of sciatica.

INTRODUCTION

Lumbar disc herniation is one of the most common sources of low back pain and radiculopathy (eg. sciatica). The knowledge of the pathophysiologic events leading to radiculopathy as a result of disc herniation was poorly understood until the 1990-ies. However, much attention has been drawn to this research field in the following years[1-6].

The nucleus pulposus is normally contained within the annulus of the intervertebral discs in the spine. After its embryological formation it normally never makes contact with the systemic circulation[7]. Immunological tolerance for nucleus pulposus is therefore considered never to develop. However, if nucleus pulposus is exposed to the immune system, for example via a disc herniation, it could thus theoretically trigger an autoimmune response. Initial support for this notion was provided by Bobechko & Hirsch in 1965[8] when they implanted harvested nucleus pulposus in the ears of rabbits, after which they studied the lymph nodes of the rabbits, and confirmed that they had grown. Further support for the auto-antigenetic properties of nucleus pulposus has subsequently been provided over the years[9-17].

One important step in the event of an autoimmune reaction is the up regulation of antigen presenting cells. One such cell is the monocyte. In the present pilot study, the aim was to assess if exposure of autologous nucleus pulposus to the host would induce an up regulation of the number of monocytes.

MATERIAL AND METHODOLOGY

Four pigs, weighing 25-30 kg, were anesthetized with an intramuscular injection of Ketalar (ketamine, Pfizer AB, Ballerup, Denmark) 15 mg/kg body weight and Dormicum (midazolam, Pharma-Hameln, Hameln, Germany) 2.4 mg/kg body weight. The pigs also received an intravenous injection of Hypnodil (methomidate chloride, AB Leo, Helsingborg, Sweden) 0.3 mg/kg body weight and Stresnil (diazempam, Dumex-Alpharma, Denmark) 3,2 mg/kg body weight. Anaesthesia was maintained by additional intravenous injections of Hypnodil 0.3 mg/kg body weight, and Stresnil 3.2 mg/kg body weight. The study was approved by the local animal research ethics committee.

Figure 1

Figure 1. The subcutaneously inserted titanium chamber. The perforated top can be dismounted and the chamber can be filled with tissue. (Bar = 5 mm)

The lumbar spine was exposed by a retroperitoneal approach[1]. The L2-3 and L4-5 intervertebral discs were incised laterally and approximately 100 mg of nucleus pulposus was harvested. The wound was sutured and the pig was placed in a prone position. Nucleus pulposus was placed in specially designed titanium chambers (Figure 1). The chambers were 5 mm high and had a diameter of 15 mm. The top could be dismounted and was perforated with 18 holes, which allowed for communication with the tissues outside the chamber. The chambers (2 in each pig) were inserted subcutaneously in the area over the lumbar spine in separate “pockets”. Two empty chambers were inserted in the same fashion to allow for analysis of the effects of the chamber per se.

After seven days the pigs were reanaestetized and sacrificed. The chambers were harvested and the perforated tops dismounted. From each chamber a total of 50µl exudate was collected and suspended in 950µl 0.1 M phosphate buffered saline (PBS) at pH 7.2.

ANALYSIS OF THE EXUDATES BY FLOW CYTOMETRY

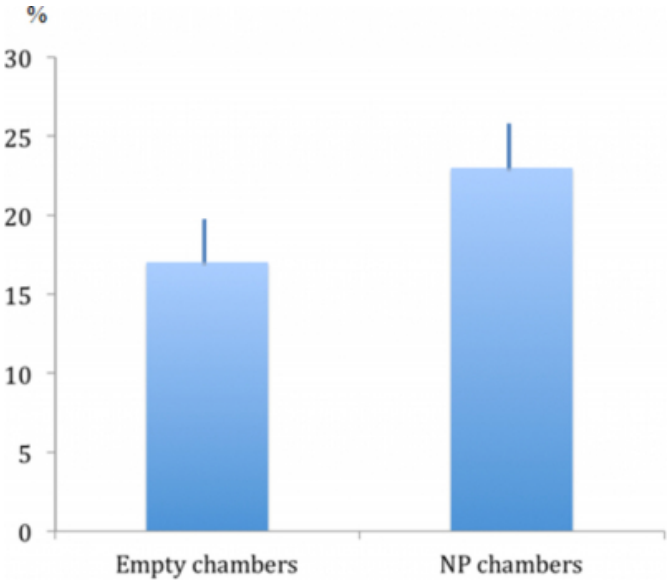
Immunophenotyping of the cells being present in the exudates was performed by enumerating the absolute number of monocytes. A total of 100µl of cell suspension was mixed with 100µl Türk staining medium (Sigma Chemical Co, St Louis, MO, USA). Of this solution, 20µl was then used for total cell counting in the Bürker chamber under a microscope.

Two hundred thousand cells were aliquoted into 1.5 ml polypropylene microcentrifuge tubes and, thereafter, suspended in 3ml PBS and centrifuged at 450 g for 10 minutes.

After decanting the supernatant 1ml of concentrated rabbit serum was added to the cells for 30 min at 25°C. After washing the cells with 3ml PBS the cells were incubated with mouse anti-pig anti-SWC3, which is an antibody directed to pig monocytes (Research Diagnostics, Flanders, USA) for 45 minutes at 25°C. After another round of washing with 3ml PBS, centrifuging at 450 g for 10 minutes, and discarding the supernatant, the cells were incubated with flourescein isothiocyanate (FITC)-conjugated F(ab’) rabbit anti mouse IgG (Serotec Ltd, Oxford, UK) mixed with 10% porcine serum, in the dark for 30 minutes at 25°C. This mix of secondary antibody and 10% porcine serum was prepared 30 minutes before the incubation. All antibodies were used in concentration of 1µg/10^6 cells. Cells were washed again in 3 ml PBS and resuspended in 500 µl PBS for flow cytometry. Flow cytometric analysis was performed on a FACScan (Becton-Dickinson, San Jose, CA, USA) using CellQuest software (Becton-Dickinson). Data are presented as mean percentage of positively stained cells as related to the unstained cells from the same sample.

Figure 2

Figure 2. The average proportion of monocytes in percent (Â±SD) in the empty chambers and NP chambers, shown as mean with standard deviation. The proportion of monocytes was statistically significantly higher in the nucleus pulposus chambers than in the empty chambers.



RESULTS

There was a significant difference ($p<0.045$, Student´s t-test) between the empty and NP chambers in the total amount of monocytes (Figure 2). While on average 17% of the cells in the empty chambers constituted monocytes, 23% of the cells in the NP chambers were monocytes.

DISCUSSION

The present pilot study demonstrated that autologous nucleus pulposus attracts more monocytes when placed subcutaneously in a titanium chamber as compared to an empty chamber.

Low back pain and sciatica are common disorders but the basic pathophysiologic events are still not fully understood[18, 19]. More than 40 years ago it was suggested that the herniation of nucleus pulposus material into the spinal canal might elicit an autoimmune reaction. It was early demonstrated that a lymphocyte response in primary lymph nodes after subcutaneous implant of nucleus pulposus (NP) in rabbits[8]. This was later confirmed by Lundskog in 1970[9]. In 1975, Elves[10] and Gertzbein[11, 12] could demonstrate leukocyte migration and in 1976 Bisla[13] demonstrated raised titers of IgM in sciatic patients. This first era exploring the autoimmune theory was concluded in 1981 when DeSilva[14] could demonstrate lymphocyte transformation but without any simultaneous increase in titers of immunoglobulins or C-reactive protein. Using more modern methods IgG and IgM was demonstrated in herniated discs by Spiliopoulu in 1994[15]. This was also later confirmed by Habtemariam[16] and Satoh[17].

The data from the present study clearly indicate that there was an increase of monocytes in the subcutaneous chambers loaded with autologous nucleus pulposus as compared to the empty chambers. This may be interpreted as monocytes are being activated and could be participating in the presentation of nucleus pulposus derived antigens to the immune system. However, one should also note that the amount of monocytes attracted to the empty chamber was, although lower than for the NP chamber, still considerably high. This may be an effect of the titanium chamber being a foreign material to the body. However, since the amount of monocytes that were attracted to the titanium chamber with nucleus pulposus was significantly higher than the amount of monocytes attracted to the empty chamber, we may assume that the present findings indicate that autologous nucleus pulposus may attract monocytes to a certain degree. Considering the implications of these findings for autoimmunity having a role in the pathophysiology for sciatica this is in contrast to a recent publication where it was found that immunoglobulins seem to be more likely to attach to cellular receptors of the nucleus pulposus cells with their Fc-part rather than their Fab-part, which would make an autoimmune mechanism less likely[20]. This may reflect the complexity of the pathophysiologic events and any role of the observed increase in monocytes for the pathophysiology of sciatica thus needs further clarification.

CONCLUSION

The data of the present study indicated that nucleus pulposus induced an up regulation of the number of monocytes. This may suggest that monocytes might play a role in the pathophysiology of sciatica.

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