

Challenging Autoimmunity As A Cause Of Sciatica.

M Bengtsson, K Olmarker

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Abstract

Although low back pain and sciatica are very common conditions the pathophysiologic mechanisms are not fully elucidated. More than 40 years ago it was suggested that since the nucleus pulposus of the intervertebral discs was isolated from the systemic circulation one might consider that there would be an autoimmune component. There have been several attempts over the years to assess this hypothesis but it is still not clear if auto-immunity is involved in the pathophysiology of, in particular sciatica. It has, for instance, been demonstrated that there are immunoglobulins in herniated nucleus pulposus tissue and that incubation of cultured nucleus pulposus cells and autologous serum will induce binding of IgG and IgM in a pig system. However, it was not clear if there was a binding of the F(ab)-region, thus implying a specific binding with possibly a pathophysiological importance, or if there was a non-specific binding of the F(c)-region. In the present study we incubated fresh nucleus pulposus tissue with commercially available F(ab)-fragments representing the F(ab)-fragments to all epitopes found in normal pig serum and commercially available F(c)-fragments. It was evident that the F(c)-fragments attached to the nucleus pulposus cells but there was no binding of the F(ab)-fragments. There is of course a possibility that IgG with specificity to the nucleus pulposus cells may be induced following immunization and is thus not present in the commercial preparation. It nevertheless seems more likely that the binding of the immunoglobulins as seen previously relates to a non-specific binding of the F(c)-part, which thus would contradict auto-immunity as a cause of low back pain and sciatica.

INTRODUCTION

Low back pain (LBP) is a clinical condition that affects a vast number of patients. It has been estimated that approximately 70% of the adult population sometimes are unable to work in their original occupation due to low back pain (1). Many of these patients also suffer from sciatica, i.e. radiating pain out into the leg. Despite its commonness it is not fully understood why a herniated disc in some patients results in pain when other patients are asymptomatic. One hypothesis is that involvement of an autoimmune mechanism could explain the observed differences in symptomatology. In conclusion, evidence of an autoimmune mechanism in sciatica has been proposed but the hypothesis has not been confirmed(2-11). Neither is there any research that has focused on the specific antigens, which might initiate this process. This might be due to earlier lack of proper methods or perhaps the fact that this research field falls between the interest of clinicians and basic scientists.

In the group it has earlier been observed that smears of pig nucleus pulposus exposed to serum from the same individual results in a binding of IgG and IgM to the surface of the NP cells(12). It was, however, not understood if these immune complexes were the result of a specific binding of the

antibodies' F(ab)-regions to certain antigens on the cell surfaces or simply a non-specific binding of the F(c)-regions, i.e. if the immune complexes could be regarded as part of a pathophysiologic process.

The aim of the present pilot project was to analyse which part of the immunoglobulins that bind to the disc cell surface.

MATERIAL AND METHODS

Nucleus Pulposus was harvested from several levels from one recently killed pig, weighing approximately 25 kg. The tissues were immediately frozen in nitrogen and sectioned at 8 µm thickness on a cryostat. Fixation was made in 50% acetone for 5 min and 100% acetone for 10 min. Endogenous tissue peroxidase activity was quenched by using 3% hydrogen peroxidase solution (H₂O₂) in PBS. The sections were incubated with goat serum for 30 min in room temperature to block unspecified bindings. The sections were then incubated with either a) peroxidase conjugated Swine IgG F(ab)-fragments (1:500) (Rockland Inc., Gilbertsville, PA, USA) or b) peroxidase conjugated Swine IgG F(c)-fragments (1:500) (Rockland Inc.) for 60 min in room temperature. The sections were finally incubated with

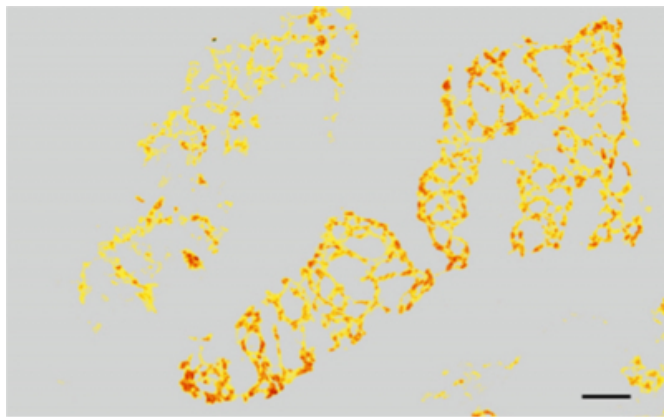
Peroxidase substrate kit DAB, SK-4100 (Vector Laboratories Inc., Burlingame, CA, USA) for approximately 7 min. Examination was performed using light microscopy.

RESULTS

Light microscopic assessment demonstrated positive results in the sections incubated with the IgG F(c)-fragments (Figure 1). This was seen as a staining of the cell population whereas there was no staining of the matrix. Sections incubated with the IgG F(ab)-fragments did not demonstrate staining of either the matrix or the cellular components.

Figure 1

Fig 1: Positive staining of the IgG F(c) fragment of the nucleus pulposus cells (red). No staining of the matrix is seen. (Bar = 100µm)



DISCUSSION

Earlier studies have shown that IgG is present in the nucleus pulposus and that it seems to be attached to the nucleus pulposus cells(9-11). It is also known that when pig nucleus pulposus is incubated with autologous serum there will be IgG present on the cell surfaces of the nucleus pulposus cells(12). However, no study has shown whether it is a specific binding of the antibodies' F(ab)-regions or simply a non-specific binding of the F(c)-regions. The data from the present study demonstrated that it is the F(c)-region and not the F(ab)-region of IgG that binds to the nucleus pulposus cells, thus implying a non-specific binding rather than a specific antibody/antigen binding.

Low back pain and sciatica are common disorders but the basic pathophysiologic events are still not entirely clear(1, 13). One mechanism that was suggested more than 40 years ago is that the herniation of nucleus pulposus material into the spinal canal may elicit an autoimmune reaction.

In 1965 Bobechko demonstrated a lymphocyte response in primary lymph nodes after subcutaneous implant of nucleus

pulposus (NP) in rabbits(2). This was confirmed in a similar study by Lundskog in 1970(3). In 1975, Elves(4) and Gertzbein(5, 7) could show leukocyte migration and in 1976 Bisla(6) demonstrated raised titers of IgM in sciatic patients. This first era exploring the autoimmune theory was however ended in 1981 when DeSilva(8) could demonstrate lymphocyte transformation but without any simultaneous increase in titers of immunoglobulins or C-reactive protein.

In 1994, using more modern methods, Spiliopoulou could show IgG and IgM in herniated discs(9). Habtemariam(10) could demonstrate IgG and IgM in herniated disc material. This was also confirmed by Satoh(11) who demonstrated IgG on the cell surface in herniated nucleus pulposus whereas These new findings indicated that autoimmunity might be of importance for the pathophysiology of sciatica. However, it has not been studied if the presence of immunoglobulins is based on a specific binding of the F(ab)-regions to certain antigens on the cell surfaces or simply a non-specific binding of the F(c)-regions.

The results of the present pilot study confirm earlier observations that IgG binds to nucleus pulposus cells and suggest that the IgG binds via its F(c)-part to F(c)-receptors on the surface of the nucleus pulposus cells. F(c)-receptors are generally found on cell surface on cells involved in the immune system such as macrophages, natural killer cells, neutrophils and mast cells(14, 15). It therefore seems likely that the previous observations of IgG being present in disc herniations and attaching to nucleus pulposus cells after incubation with autologous serum is not based on the presence of specific antigens on the nucleus pulposus cell surfaces. Instead, this is probably more likely related to a non-specific binding of the F(c)-region of the IgG.

One limitation of the study is that it was performed on nucleus pulposus material from only one pig. However, since there was binding of the F(c)-fragments one would not expect that the addition of more individuals would change the outcome of this part of the study. Another limitation is that commercially available preparations of F(ab)-fragments were used. The rationale was that the population of the F(ab)-fragments would represent the immunoglobulins with specificity to all epitopes, as normally found in pig serum. Since there was no binding of these F(ab)-fragments it seemed that normal serum did not contain any IgG with specificity to epitopes on the nucleus pulposus cells. There is, however, a possibility that IgG with an F(ab)-region specific to antigens on the surface of the nucleus pulposus cells may be obtained if the pig is first immunized to its own

nucleus pulposus. This will be considered in future studies.

In conclusion, since it seems to be the F(c)-and not the F(ab)-region of IgG that binds to the surface of nucleus pulposus cells the data of the present study indicate that it is less likely that the pathophysiology involves detection of specific antigens on the cell surface of nucleus pulposus cells by the immune system, thus not favouring the autoimmune hypothesis.

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Author Information

Magnus Bengtsson, MD

Department of Medical Chemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg

Kjell Olmarker, MD PhD

Department of Medical Chemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg