Yonsei Medical Journal
Vol. 22, No. 1, 1981

Auto-immune Response in Degenerated Lumbar Disk*

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The serum level of Ig G, Ig M, Ig A, Ig D and C_3 were determined by a Hyland Immunodiffusion plate in 60 patients and 29 normal healthy controls. Immunoglobulin G level was increased only in patient's with herniated nucleus pulposus.

We also could demonstrate high levels of Ig G in group I, the ruptured nucleus cases, but there were no differences in the levels of other immunoglobulins and C_3 in other groups.

In the Ochterlony gel diffusion test for the detection of humoral antibody for the nucleus pulposus, only 4 cases gave precipitation lines using antigen excised from nucleus pulposus. In 2 cases high levels of Ig G were observed. All 89 patients gave negative precipitation lines using proteoglycan as antigen.

Key Words: Disk, Autoimmunity.

The low back pain is a symptom complex occuring in various conditions in the low back and include spondylogenic, discogenic, neurogenic, vascular, viscerogenous and psychogenic causes.

The main cause in the active working age is usually thought to be a disk problem, and it is summerized in three ways in the view point of pathophysiology.

The first cause is direct mechanical compression of the nerve root, secondly the nerve root is compressed by swollen soft tissue due to circulatory disturbance and thirdly the disk degeneration is caused by auto-immune response which irritates nerve tissue.

The human intervertebral disk is a dynamic and specialized connective tissue structure that has three main structural components—the vertebral cartilagenous end plate, annulus fibrosus and nucleus pulposus.

The nucleus pulposus has evolved to absorb and redistribute forces applied to the vertebral column and is made up of a matrix, collagen and cells. These components vary in certain aspects from other connective tissue to enable them to undertake their specialized function.

After embryological development the nucleus pulposus of the intervertebral disk is enclosed

Received May 12, 1981

*This research was supported by CMB Grant (1977) (China Medical Board) in New York (Grant No. 77-8).

within the annulus and has no vascular contact with the blood system (Naylor, 1970).

Disk degeneration with or without herniation is the most common cause of low back pain. In the past 40 years considerable study has been done to understanding low back pain, but the cause of the disk degeneration is still obscure and speculative (Scott, 1955; Brown *et al.*, 1957; and Naylor, 1970).

There are some studies concerning disk degeneration in biochemical and auto-immunological basis.

It has been suggested by Naylor (1962) that low back pain may have an auto-immune basis. Bobechko and Hirsh (1965) have suggested that hypervascularization in the region of a degenerated disk may expose disk tissue to contact with the immunological apparatus and stimulate an autoimmune reaction.

Since the protruded disk in younger men demonstrates a loss of water, an increase in collagen and protein and a decrease in mucopolysaccharides, which is consistent with what is found in nonprotruded disks from older individuals, it is felt by some that disk degeneration represents a premature aging process.

On the other hand some other workers suggest that neither age nor trauma are the sole factors and some causes responsible for a basically spontaneous onset should be considered.

This auto-immunity as an etiological factor in the pathogenesis of degenerated disk disease has been considered by several authors (Bobechko and Hirsch, 1965; Naylor, 1962; Elves *et al.*, 1975; and Pankovich and Korngold, 1967).

Being devoid of vascularity, the nucleus pulposus is not in contact with the body's immune mechanisms.

If the nucleus pulposus ruptures into the spinal canal, it will contact the immune system.

This mechanism does not explain the etiology of degenerated disk disease but it may account for the prolongation of clinical symptoms.

From the immunological view point, the indirect methods of showing cellular immunity, i.e. the leukocyte migration test (Federlin *et al.*, 1971) and lymphocyte microstimulation test (Lockshin *et al.*, 1975), have brought new interest to the auto-immune theory.

In this investigation, we attempted to trace the change of the immunoglobulins in the serum and detection of the antibody in the nucleus pulposus, and what is the possibility of prevention of treatment of low back pain on an auto-immune basis. We reviewed 60 patients who complained of low back pain with back surgery compared with 29 healthy persons.

MATERIALS AND METHODS

Materials

We selected 60 patients, 32 males and 28 females hospitalized in the Department of Orthopedic Surgery, Yonsei University College of Medicine from September 1978 to January 1981. There were 33 patients diagnosed as having herniated nucleus pulposus (H.N.P.), clinically, radiologically and pathologically, who had surgery in the form of laminectomy or diskectomy and interbody fusion (Kim *et al.*, 1979).

There were 27 patients with low back pain diagnosed as having spondylolysis or spondylolisthesis who had interbody fusion.

These patients were divided into 3 groups as follows:

Group 1: There were 22 patients with low back pain whose plain and myelographic radiological finding showed protrusions of nucleus pulposus and osteophytes. The rupture and degeneration of disks were confirmed at surgery.

Group 2: There were 19 patients with low

back pain whose radiological findings showed osteophytes without any rupture of disks. The degeneration of the disk was confirmed at surgery.

Group 3: There were 19 patients who had low back pain only. This included some spondylolysis or spondylolisthesis without evidence grossly of rupture or degeneration of the disk. For the controls, we selected 29 persons who had no history of back pain.

Methods

1. Serum immunoglobulins and C_3: We obtained 10 ml of blood at surgery, and the Ig G, Ig A, Ig M, Ig D, and C_3 in the serums of 89 persons were determined by the Hyland Immuno-Plate 1 Radial Immunodiffusion test and the levels are reported in mg/dl (W.H.O. Unit).

2. Standard Ochterlony Gel Diffusion Test: It was performed for the detection of antibody for the antigens from excised nucleus pulposus and the proteoglycans of uncleus pulposus.

Preparations of Antigen (excised from nucleus pulposus; Elves *et al.*, 1975): The nucleus pulposus was removed at operation and frozen until use. While still frozen, it was cut into thin slices and homogenized in TC 199, and incubated at 37°C for one hour to allow autolysis to occur. The homogenate was then centrifuged at 2000 r.p.m. for one hour. The viscous and slightly cloudy supernatant fluid was decanted off. This extract was supplemented, using 20 per cent decomplemented fetal calf serum.

Preparation of Proteoglycans (Sajera and Hascall, 1969): The nucleus pulposus removed at operation was frozen. While still frozen, it was minced into thin slices by scissors and homogenized in TC 199. It was also allowed to interact with 3 M $MgCl_2$ for 48 hours. The insoluble residues were removed by centrifugation. The supernatant was dialyzed against distilled water for 48 hours until all $MgCl_2$ was removed. The crude proteoglycans were prepared for the test.

RESULTS

The age and sex distributions in this study are shown in Table 1. We tested 89 persons aged between sixteen to sixty-one, 52 males and 37 females.

In this test, any differences of Ig G, Ig M, Ig A, Ig D and C_3 were not observed in spondylolysis or spondylolisthesis, compared to the normal healthy controls ($P>0.05$). There is no significant difference in Ig A, Ig D, and C_3 between H.N.P. and healthy controls, but Ig G in H.N.P. was significantly increased over the normal controls ($p<0.05$) as shown in Table 2. The mean age for H.N.P. was 35.7 years old.

In regarding age (Table 3), increases of Ig G and Ig M were observed in the age group of 40-49 ($p<0.05$), which is highly significant.

The age distribution of each of 3 groups is shown in Table 4. In group 1, there were 22 patients who had neurologic lesions on physical examination and positive radiologic defects on myelography. In this group, the immunoglobulin level of Ig G was significantly higher than in the controls ($p<0.05$). We were unable to find any significant difference in other

Table 1. Age and sex distribution

Age	H.N.P(M/F)	Spondyl.(M/F)	Control(M/F)
10-19	3 (3/0)	2 (0/2)	4 (4/0)
20-29	6 (6/0)	8 (4/4)	10 (7/3)
30-39	10 (6/4)	7 (4/3)	5 (4/1)
40-49	10 (4/6)	5 (1/4)	6 (3/3)
50-59	4 (2/2)	5 (1/4)	4 (3/1)
Total	33 (21/21)	27 (10/17)	29 (21/8)

* H.N.P. : Herniated Nucleus Pulposus
* Spondyl. : Spondylolysis and spondylolisthesis

Table 2. Mean value of immunoglobulins and C_3

	H.N.P.	p value	Spondylo.	p value	Control
Ig G	1419.3±107.5	p<0.05	1116.5±44.3	p>0.05	1188.5± 42.1
Ig M	123.7± 4.2	p<0.1	103.7± 5.5	p>0.05	107.1± 8.2
Ig A	185.2 ± 15.2	p>0.05	200.6±12.5	p>0.05	188.4± 13.1
Ig D	2.5± 0.4	p>0.05	3.2 ± 0.4	p<0.05	1.9 ± 0.3
C_3	108.2 ± 8.4	p>0.05	108.8 ± 8.3	p>0.05	103.2 ± 7.2

Table 3. Values of Ig G and Ig M in the group of 30-49

		H.N.P	Control	p value
Ig G	30-39	1365.2	1365.2	p>0.05
	40-49	1444.5	1162.8	p<0.05
Ig M	39-39	117.6	118.2	p>0.05
	40-49	129.3	108.9	p<0.05

Table 4. Age distributions in the 3 groups

	Group 1	Group 2	Group 3	Control
10-19	2	2	1	4
20-29	4	2	8	10
30-39	8	4	5	5
40-49	6	8	1	6
50-59	2	3	4	4
Total	22	19	19	29

Table 5. Values of Ig G, Ig M, Ig A, Ig D and C_3 in group 1

	Group 1	Control	p value
Ig G	1408.6	1185.5	p<0.05
Ig M	115.0	107.1	p>0.05
Ig A	190.2	188.4	p>0.05
Ig D	2.7	1.7	p<0.05
C_3	107.1	103.2	p>0.05

Table 6. Values of Ig G, Ig M, Ig A, Ig D and C_3 in group 2

	Group 2	Control	p value
Ig G	1225.0	1185.5	p>0.05
Ig M	116.7	107.1	p>0.05
Ig A	194.8	188.4	p>0.05
Ig D	2.8	1.7	p<0.05
C_3	112.0	103.2	p>0.05

Table 7. Values of Ig G, Ig M, Ig A, Ig D and C_3 in group 3

	Group 3	Control	p value
Ig G	1216.9	1188.5	p>0.05
Ig M	114.8	107.1	p>0.05
Ig A	191.2	188.4	p>0.05
Ig D	2.9	1.7	p<0.05
C_3	107.2	103.2	p>0.05

immunologic levels and in C_3 (Table 5).

In this group, herniated disks and gross degeneration of the nucleus pulposus were found at surgery in all patients. In 19 patients with low back pain, positive radiologic defects and no evidence of rupture of the disk, there were slight increases of Ig M compared with normal healthy controls (Table 6).

We could not observe any differences between group 3 and the controls. These 19 patients

Table 8. Result of Ochterlony Gel Diffusion Test

Sex/age	Disease	Ig G	Ig M	Ig A	Ig D	C_3
m 23	Spondylolysis	1240.8	103.6	208.0	0.5	102
m 21	H.N.P	1024.1	114.2	221.0	0.8	102
m 16	H.N.P	1654.4	107.8	102.7	0.3	94
m 37	H.N.P	1633.5	120.3	101.4	1.8	113

had only low back pain without evidence of disk degeneration or herniation (Table 7).

The average durations of back pain were 1 year and 2 months in group 1, 11 months in group 2, and 2 years and 3 months in group 3. Ig D was significantly increased in all three groups compared to controls (Table 5, 6, 7).

Study of Ochterlony Gel Diffusion Test: We could observe only 4 persons who gave positive results on using the antigens excised from the nucleus pulposus, but there were no precipitations on using the proteoglycans from nucleus pulposus as antigens in all 89 patients. The history of back pain, positive radiology, pathology, surgical findings, and the values of Ig G, Ig M, Ig A, Ig D, and C_3 in the above mentioned 4 patients were shown in Table 8. Two persons showed high levels of Ig G without any change of other immunoglobulins and C_3. Two persons was observed slightly increased Ig M. All four persons had a history of back pain, disk degenerations and mild or severe rupture of nucleus pulposus.

DISCUSSION

Since the classical paper by Mixter and Barr in 1934, considerable knowledge has been gained regarding the role of intervertebral disks in the pathogenesis of low back pain, but the etiology of low back pain has not been solved. Many theories have been advanced for disk degeneration and herniation. Brown (1971) speculated that the pathologic process of the disc degeneration is the result of rapid depolymerization of the acid mucopolysaccharide. Trauma (Brown *et al.*, 1957), ACTH (Naylor, 1962) cell-mediated immunity (Bobechko and Hirsch, 1965) and ageing process were suggested for the etiology. Bocechko and Hirsch (1965) were able to produce cellmediated immunity to the nucleus pulposus and could set up a chronic inflammatory reaction in rabbits. Elves *et al.* (1975) and Gertzbein *et al.* (1975) were able to demonstrate cellular immunity in patients with disk prolapse by leukocyte migration inhibition tests. The nucleus pulposus is devoid of blood supply after 8 years of age and must be exposed to the reticulo-endothelial system for the induction of autoimmunity. It has been shown that repeated biochemical forces may lead to loss of cohesion between bundles of annulus, leading to fissuring. These fissures will be repaired by the ingrowth of granulation tissue which leads to vascularization, thus initiating the auto-immune phenomenon. In some cases micro-fractures of the vertebral endplate or Schmorl's nodes may also induce the auto-immune phenomenon due to vascularization from the vertebrae.

The study of Naylor *et al.* (1975) showed a significant enhancement of Ig M and Ig G in those with lumbar disk prolapse. These suggest that either a nonspecific antigenic process or stimulation of an antibody humoral system is a factor in the development of lumbar disk

prolapse.

It would appear, further, that there is a proportion of the population with an unstable or hyperactive reticuloendothelial system and possibly that in susceptible individuals some triggering stimulus or minor trauma may excite the reticuloendothelial system to produce excess antibodies to disk protein.

The serum immunoglobulin and C_3 analysis by Hyland immuno-diffusion plate revealed that Ig G levels were significantly higher ($p<0.05$) as compared to the control (Table 2 and 3). These levels are not explained by the age of the patients except in the 40-49 age group. We were not able to confirm to findings of Naylor (1975) and Bisla *et al.* (1976). Naylor (1975) reported that the serum level of Ig M was significantly increased in the whole range of groups, but Ig G, Ig M and Ig A were increased in the specific age group of 30-39. Bisla *et al.* (1976) reported the serum level of Ig M, irrespective of the age of the individual, was significantly higher in patients with backache than in asymptomatic patients. But, in our experiment only the Ig G level was highly increased in prolapsed nucleus pulposus patient and, in regard to the age 40-49 group, only Ig G and Ig M were significantly higher than in the control. The serum level of Ig D was increased in all groups, but we were not able to explain the result.

It is not clear what the nature of the immunizing antigen is. The nucleus pulposus is almost acellular and therefore cell associated antigens can probably be ruled out. Another possibility is that the proteoglycan of the matrix is antigenic. Recently, cell mediated immunity to laryngeal cartilage proteoglycan has been demonstrated in two patients suffering from relapsing polychondritis. (Rajapakse and Bywalers, 1974). Soluble proteins extracted from disc materials removed at operation for disk herniation were examined for the detection of humoral antibody in the tested serums. Ochterlony gel diffusion techniques were used. A soluble antigen and proteoglycans extracted from the nucleus pulposus were applied to the central well for a precipitation line.

The sera from 89 patients tested with disk antigens formed a precipitation line indicating the presence of an anti-globulin to disk material. The Ochterlony gel diffusion technique did not demonstrate the presence of humoral antibody in our tests except in 4 cases using the antigen excised from the nucleus pulposus, but we could not demonstrate any humoral antibody, using the proteoglycans as an antigen.

Among 4 patients who showed precipitation lines, two patients showed high levels of Ig G.

This finding is compatible with the result of increased Ig G in Naylor's experiment (1975).

SUMMARY

The serum levels of Ig G, Ig M, Ig A, Ig D and C_3 were determined by a Hyland Immunodiffusion plate in 60 patients and in 29 normal healthy controls.

Immunoglobulin G level was increased in herniated nucleus pulposus patients but spondylolysis and spondylolisthesis were observed as in normal range. We divided 60 patients into 3 groups according to the serverity of disk symptoms, including physical, radiological and surgical findings. We could demonstrate high levels of Ig G in group 1, the ruptured nucleus cases, but there were no differences in the levels of other immunoglobulins and C_3 in other groups. For the detection of humoral antibody for the nucleus pulposus, we used the Ochterlony gel diffusion test. As antigens, we used two types: one was Ag from excised nucleus pulposus and the other was proteoglycan from nucleus pulposus. Only

4 cases gave precipitation lines using antigen excised from nucleus pulposus. In 2 cases high levels of Ig G were observed. All 89 patients gave negative precipitation lines using proteoglycan as antigen.

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