Ankylosing Spondylitis, HLA-B27 and *Klebsiella* – An Overview: Proposal for early diagnosis and Treatment.

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Abstract: *Ankylosing spondylitis (AS) is a potentially disabling rheumatic disease for which no curative treatment has yet been discovered.*

An extensive computer-based and manual search was undertaken to evaluate the role of microbes in the pathogenesis of AS. All together 147 papers were scrutinised. A total of 24 studies carried out on 1330 AS patients and 1191 healthy controls involving 15 different countries showed significantly elevated Klebsiella antibodies in AS patients when compared to controls. Molecular analysis has shown that Klebsiella microbes possess antigens which cross-react with self-antigens, such as HLA-B27 and spinal collagens.

Diagnostic criteria have been developed in which a person who is HLA-B27 positive and has clinical and laboratory evidence of an inflammatory backache for at least three months is proposed to have pre-AS. A specific elevation of anti-Klebsiella antibodies would confirm the diagnosis. A proposal for an early treatment using anti-Klebsiella measures is suggested.

So far, apart from Klebsiella no other microbes have been shown to have a link with the development of AS. It is suggested that identifying and treating patients with Klebsiella reactive arthritis/pre-AS could involve the use of anti-Klebsiella measures, such as antibiotics and low starch diet together with immunosuppressive drugs in an endeavour to prevent the irreversible sequelae of established AS.

Keywords: Ankylosing Spondylitis, HLA-B27, *Klebsiella,* new diagnostic marker.

INTRODUCTION

Ankylosing spondylitis (AS) is the main disease among a group of inflammatory arthritides collectively known as "spondyloarthropathies." It is a chronic disease usually starting in males and less commonly in females, between the ages of 15 and 40 years, and sometimes as young as 8 years (1). It commonly affects the lower back, especially the sacroiliac and lower spinal joints. Less frequently other large joints such as the hips, knees and ankles are involved. The points of attachments of

tendons, ligaments and joint capsules, also known as the "entheses" are involved in the pathology of AS. Non-articular manifestations include eye problems such as uveitis or iritis which occurs in 25% of AS patients and occasionally aortitis and pulmonary fibrosis (2). The prevalence of complete AS appears appears to be in the region of 0.25 to 1% with a peak of 2% in Northern Norway (3). Many AS patients have functional disabilities which can lead to economic difficulties (4).

Many attempts have been made to find the causative factor for AS and identify the early stages of the disease so that suitable treatment can be started before undesirable permanent pathological changes in the spine and elsewhere have occurred.

THE AETIOLOGICAL ROLE OF HLA-B27

The association between AS and HLA-B27 remains to this day one of the most common examples linking susceptibility to develop an arthritic disease to the genes of the "Major Histocompatibility Complex" or MHC for short. The MHC complex is encoded on chromosome 6 and everybody has 2 such MHC genes, one inherited from mother and the other one from father. This remarkable association between HLA-B27 and AS was reported nearly 30 years ago from two centres, one in Los Angeles and the other one from London (5,6) and has been shown to be present in most populations throughout the world. The frequency of HLA-B27 in AS patients ranges from 81 to 96% while its frequency among the healthy populations is between 4 and 8%. Rats into which the HLA-B27 gene has been transmitted develop a chronic inflammatory disease which resembles AS, such as a stiff tail, whilst control rats given another gene such as HLA-A2 do not develop the disease (7). Rats having the largest numbers of such genes have a higher frequency of conditions resembling AS (8). These animal experiments clearly indicate the importance of the HLA-B27 gene in the occurrence of AS.

The prevalence of AS correlates with the presence of HLA-B27 in the majority of the ethnic groups studied. For example, African Blacks of unmixed ancestry lack HLA-B27 genes and AS is very rare among them. On the other hand, certain North American Athabascan Indian tribes who have a high frequency of HLA-B27 also have a high prevalence of AS (9). The fact that nearly all races show an association of AS with this genetic marker suggests that HLA-B27 is somehow involved in the disease development. Nevertheless, it must be noted that over 80% of HLA-B27 positive individuals do not develop symptoms of AS. This means that in the USA there are about 24 million individuals who carry HLA-B27 and some 5 million of these have some symptoms of AS. In the UK there are about 5 million individuals who possess HLA-B27 and approximately one million of them have some symptoms of AS. It would appear, there are in the world over 20 million individuals who suffer from "B27 disease."

There are at least 25 different sub-types of HLA-B27 and B*2705 would seem to be the ancestral subtype from which the others have evolved (10). Studies of the HLA-B27 molecule by two-dimensional gel electrophoresis (11), DNA sequencing (12) and restriction fragment length polymorphism (13) have all shown no molecular differences in this genetic marker between AS patients and healthy controls. Furthermore, using HLA-B27 specific monoclonal antibodies, no disease associated variant of HLA-B27 has been observed in AS (14). This means that the HLA-B27

molecule is the same in AS patients as it is in healthy HLA-B27 individuals who have no symptoms of AS.

Restriction fragment length polymorphism has failed to confirm the presence of any additional genetic material of relevance to disease susceptibility in AS. For example, no difference was detected in the frequencies of "tumour necrosis factor" genes between AS patients and healthy controls (15) and no association was observed between IL-10 promoter gene polymorphisms and susceptibility to AS (16). Differential linkage disequilibrium analysis with HLA-B27 subtypes suggests that B27 itself remains the primary gene for AS susceptibility and other closely related MHC genes are not involved in the pathogenesis of the disease (17). Thus the property mediating susceptibility to AS or "B27 disease" appears to lie in the features of the serologically and biologically determined HLA-B27 molecules, which are common to nearly all its subtypes. Moreover, most of the HLA-B27 negative individuals who develop AS were shown to belong to those carrying HLA-B7 cross reactive group (B-7 CREG) genes and might present as milder forms of the disease (18).

THE AETIOLOGICAL ROLE OF ENVIRONMENTAL FACTORS

In spite of the strong genetic link of HLA-B27 with AS, other non-genetic, environmental factors, particularly microorganisms, could be involved in triggering the disease:

*   The concordance rate for AS in identical twins is between 35% (19) and 75% (20). The concordance rate measures the percentage of the second twins having the disease when the first twin suffers from the same condition.

*   The episodic nature of AS with relapses and remissions weakens the possible role of a pure and isolated genetic factor acting alone in the development of this condition (21).

*   Rats into which the HLA-B27 gene has been transferred when raised in germ-free conditions do not develop AS and this strongly suggests that the bowel microbes play an important role in the pathogenesis of "B27 diseases" (22).

*   Variations in the genetic makeup are not reflected in the differences of certain clinical parameters in AS patients (23).

SEROLOGICAL EVIDENCE FOR *KLEBSIELLA* INVOLVEMENT IN "AS"

Infection has long been regarded as a likely cause of AS since evidence linking this disease with chronic prostatic infection emerged in the 1950's (24). It was nearly 20 years later, in 1976, when infection with *Klebsiella* microbes was first implicated in the possible causation of AS (25). The evidence linking *Klebsiella* microbes to AS have involved a number of experimental and clinical studies:

*   Elevation in the total serum IgA (26-28), total secretory IgA (29) as well as *Klebsiella* specific IgA (30) has been observed in AS patients during active phases of the disease. Moreover, a significant

correlation was found between total secretory IgA and anti-*Klebsiella* antibody concentrations in Spanish AS patients (31).

* Increased antibody titres against *Klebsiella* but not to other microbes including *E.coli, Yersinia, Salmonella, Pseudomonas, Proteus* and anaerobic bacterial species (32-35) have been detected in the sera of 1330 AS patients from 15 different countries when compared to 1191 healthy controls (Table 1). Furthermore, 671 AS patients from 8 different countries were compared to 466 patients having other arthritic diseases, such as rheumatoid arthritis or osteoarthritis, and again were found to have elevated levels of antibodies against *Klebsiella* microbes (Table 2).

Table 1: Geographical Distribution of Anti-*Klebsiella* Antibodies in AS Patients Compared to Healthy Controls (HC)

Country	Location	Number of AS Patients	Number of HCs	P-value	Reference
England	London	107	110	<0.01	[32]
England	London	35	60	<0.001	[34]
England	London & Stevenage	97	25	<0.005	[45]
England	London	36	26	<0.001	[54]
England	London	100	50	<0.001	[76]
England	London	65	57	<0.005	[133]
England	London	40	40	<0.001	[134]
England	London	66	51	<0.001	[135]
USA	Chapel Hill	24	90	<0.001	[42]
Scotland	Glasgow	59	35	<0.001	[136]
Scotland	Edinburgh	14	14	<0.05	[137]
Slovakia	Piestany	20	20	<0.05	[138]
Canada	Winnipeg	31	15	<0.001	[139]
China	Beijing	60	45	<0.001	[140]
Netherland	Amsterdam	34	34	<0.001	[141]
Turkey	Ankara	40	40	<0.001	[142]
Japan	Otsu	52	50	<0.001	[33]
Finland	Helsinki	184	100	<0.001	[74]
Finland	Helsinki & Turku	84	100	<0.001	[116]
Mexico	Mexico DF	44	40	<0.0001	[143]
Germany	Nuremberg & Kiel	41	95	<0.01	[144]
Taiwan	Taichung	52	51	<0.001	[145]
India	Madras	20	15	<0.0005	[146]
Russia	Moscow	25	28	<0.0005	[147]
Total number of subjects		**1330**	**1191**		

* These antibody observations were demonstrated by independent groups, using a number of different immunological methods and coming from many countries throughout the world.

* *Klebsiella* antibody titre was found to be significantly higher in the serum than in the synovial fluid of a group of Finnish AS patients, thereby suggesting that there was no intra-articular production of antibodies to this microbe (35). This strongly suggests that the *Klebsiella* microbes causing AS are not in the joints but in the gut.

However in a recent study from Canada, the failure to find a significant difference in anti-*Klebsiella* between AS patients and healthy subjects was not unexpected, as no correlation was carried out between disease activity status and the antibody titres (36). The presence of inflammation is necessary in defining the disease activity in AS patients, based on the estimation of the "erythrocyte sedimentation rate" (ESR) and "C-reactive protein" (CRP), and it is only in those patients that elevated anti-*Klebsiella* antibody will be observed (33).

Table 2: Geographical Distribution of *Klebsiella* Antibodies in Patients with AS Compared to those with Other Rheumatic Diseases (ORD).

Country	Location	Number of AS	Number of ORD	P-value	Reference
England	London & Stevenage	97	25RA	<0.005	[45]
England	London	100	50RA	<0.001	[76]
England	London	65	43RA	<0.001	[134]
			21PsA	<0.001	
England	London	66	31RA	<0.001	[135]
Spain	Barcelona	84	22RA	<0.0001	[31]
			41NIA	<0.005	
Canada	Winnipeg	31	18RA/OA	<0.001	[139]
China	Beijing	60	28RA	<0.02	[140]
Japan	Otsu	52	50RA	<0.001	[33]
Finland	Turku	8	10RA	<0.05	[35]
Netherland	Amsterdam	34	25RA	<0.001	[141]
Germany	Lubeck & Kiel	54	24RA	<0.01	[144]
			20ReA	<0.01	
			24SLE	<0.01	
			24PsA	<0.01	
India	Madras	20	10ReA	<0.05	[146]
Total number of subjects		**671**	**466**		

*RA: Rheumatoid Arthritis; PsA: Psoriatic Arthritis; NIA: Non-Inflammatory Arthropathy; OA: OsteoArthritis; ReA: Reactive Arthritis; SLE: Systemic Lupus Erythematosus.

EVIDENCE FOR CROSS-REACTIVITY BETWEEN *KLEBSIELLA* AND HOST ANTIGENS

Immunological and molecular cross-reactivity between HLA-B27 or other self-antigens and *Klebsiella* microbes have been demonstrated in several independent studies:

(1)   Sera from rabbits immunised with HLA-B27 positive lymphocytes reacted with antigenic extracts of *Klebsiella, Salmonella, Shigella* and *Yersinia* microorganisms indicating the presence of shared cross-reactive antigens between HLA-B27 and these microbes (25,37).

(2)   Specific anti-B27 human tissue typing sera were found to bind to *Klebsiella* antigens more readily than to other tissue typing sera, which were specific for other HLA antigens (38).

(3)   Monoclonal anti-B27 antibodies were found to bind specifically to *Klebsiella, Shigella* and *Yersinia* antigens (39).

(4)   Anti-HLA-B27 monoclonal antibody (M2) bound specifically to 60 and 80 Kd components of *Klebsiella* whereas no such reactivity was demonstrated by 5 other monoclonal antibodies (40).

(5)   Although both *Klebsiella* and *Yersinia* microbes possess antigens cross-reacting with HLA-B27, AS patients have significantly elevated levels of antibodies only to *Klebsiella* but not to *Yersinia* microbes (41).

(6)   Studies from La Jolla in California have shown that there is molecular identity between a 6 amino acid sequence "QTDRED" which is present in HLA-B27 molecules (residues 72-77) and *Klebsiella* nitrogenase reductase enzyme (residues 188-193) (Figure 1). Significantly increased levels of antibodies to a synthetic peptide obtained from *Klebsiella* nitrogenase and containing this similarity sequence were found in American AS patients when compared to healthy controls (42).

(7)   Antibodies against *Klebsiella* nitrogenase peptides containing the "QTDRED" sequence, bind more significantly to the synovial tissues taken from American AS patients when compared to those with other inflammatory diseases (43).

(8)   Sera from rabbits immunised with *Klebsiella* microbes were able to distinguish HLA-B27 positive lymphocytes from either AS patients or healthy controls when compared to lymphocytes obtained from HLA-B27 negative individuals (44). This demonstrates that there is no difference between the HLA-B27 molecule found in AS patients or in healthy HLA-B27 positive individuals and for disease to occur there must be the intervention of the *Klebsiella* microbe. This immediately suggests a possible therapeutic intervention, if the

Klebsiella microbe could be somehow reduced in quantity or even eliminated, it might be of benefit to AS patients. Since gut bacteria grow on the residues of food entering the large bowel from the small bowel, reduction in the consumption of large molecules such as starch could reduce the activity of the disease.

(9)   Another molecular similarity has been demonstrated between the "DRDE" amino acid sequence (positions 596-599) present in the PulD secretion proteins of *Klebsiella* pullulanase enzymes and the "DRED" sequence (positions 74-77) (Figure 1) present in the HLA-B27 molecules. Antibody levels against each of these peptides were found to be elevated in English AS patients from London when compared to healthy controls (45).

(10)   There are also similarities between *Klebsiella* and collagen tissues. Gly-x-pro repeating sequences were found in the PulA components of *Klebsiella* pullulanase, which shows molecular similarity with type I, type III and type IV collagens, and such collagens are found in the tendons, spine, large joints and uvea. This could explain why AS involves the spine, large joints, tendons and also eye tissues, thereby accounting for uveitis and iritis (45).

(11)   A significant elevation in the levels of IgA antibodies to the synthetic peptides containing "DRDE" or "DRED" amino acid sequences of the *Klebsiella* PulD proteins or HLA-B27 molecules were observed in Japanese AS patients when compared to patients with rheumatoid arthritis or healthy controls (33).

(12)   Significant elevation of the secretory IgA2 antibodies against type I, type III and type IV collagens, cross-reactive with *Klebsiella,* were observed in Japanese AS patients when compared with healthy individuals (46).

(13)   AS patients were found to have increased antibody concentrations of IgG and IgA classes to KP2 components of the *Klebsiella* nitrogenase enzyme when compared to controls (47).

(14)   In a study reported in 2003, IgG antibodies from AS patients were found to be significantly cytotoxic to HLA-B27 peptide bearing cells, as shown by increased percentage lysis for sheep red blood cells coated with HLA-B27 peptide QTDRED, when compared to sera obtained from patients with rheumatoid arthritis (p<0.001) or sera obtained from healthy blood donors (p<0.001) (48). These results would appear to indicate that the pathological damage in AS patients is caused by anti-*Klebsiella* antibodies.
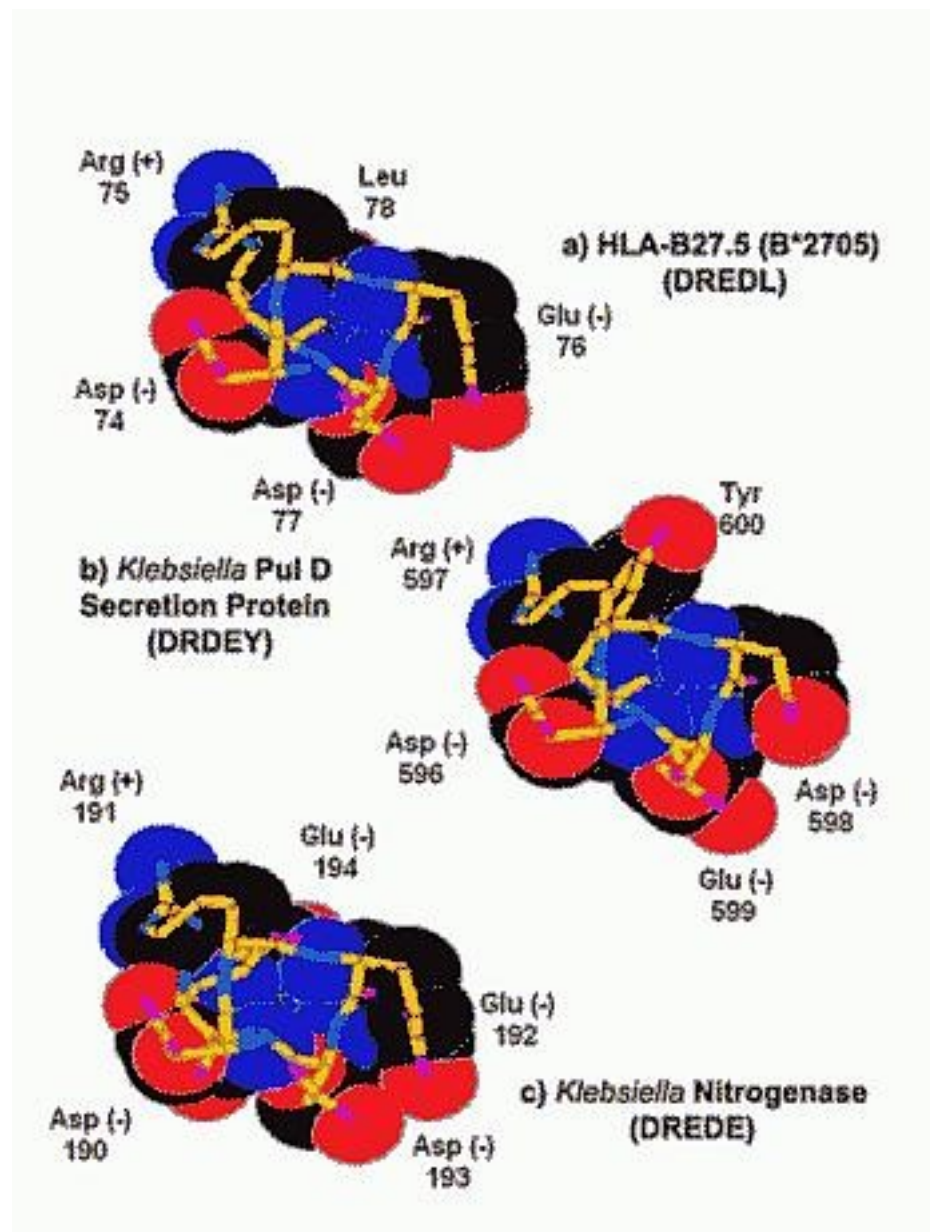
FIGURE 1: Molecular similarities between peptides from nitrogenase reductase and pullulanase enzymes of *Klebsiella pneumoniae* compared to the HLA-B27 molecule.

GUT AS THE MAIN SOURCE OF *KLEBSIELLA* INFECTION IN "AS"

It is well known that occasionally a "reactive arthritis" will occur after a gut infection caused by *Yersinia, Shigella, Salmonella* and *Campylobacter* microorganisms (49). *Klebsiella* microbes belong to these groups of Gram negative microorganisms. Furthermore, there is extensive evidence of an association between gut lesions and AS, and the presence of *Klebsiella* would appear to be the most likely triggering agent acting across the gut mucosa:

(1)   Many close relatives of AS patients appear to have an inherited abnormality that leads to sub-clinical gut inflammation (50).

(2)   HLA-B27 positive individuals suffering from "inflammatory bowel disease" (IBD) such as Crohn's disease or ulcerative colitis have a significantly greater chance of developing AS than HLA-B27 positive individuals not having IBD (51).
In a population study , it was observed that AS occurred more frequently in patients with newly diagnosed IBD, where prevalence of AS was reaching nearly 3% in ulcerative colitis and 6% in Crohn's disease.(52).
In a Belgian study it was found that more than 50% of AS patients were having asymptomatic IBD when investigated by colonoscopy (53).

(3)   Patients with AS, as well as those having Crohn's disease and to a lesser extent ulcerative colitis but not those with rheumatoid arthritis or healthy controls have significantly higher titres of anti-*Klebsiella* antibodies, indicating the possibility of a microbial link between AS and these IBD's (34).

(4)   Patients with AS and Crohn's disease were found to have elevated levels of IgM, IgG and IgA class specific antibodies to *Klebsiella* and to type I, III, IV and V collagens. A positive correlation was also observed between *Klebsiella* and collagen antibody levels in these patients. This suggests that AS in HLA-B27 negative patients may also be caused by *Klebsiella.*

(5)   Macroscopic and microscopic inflammation in the bowel mucosa (55) together with enhanced gut permeability occurs in AS patients (56,57,58).

(6)   Using a radioactive technetium leucocyte labelling technique, 48% of HLA-B27 patients with AS, showed bowel inflammation; all had active joint disease, whereas no patients with inactive disease had a positive uptake of labelled leucocytes (59).

(7)   Temporal relationships between the acquisition of *Klebsiella* in the faeces and exacerbation of symptoms were observed in 2 longitudinal studies carried out on English AS patients (60,61). Another group from Germany found that among 4 *Klebsiella* isolates and 42 different serotypes, only *Klebsiella oxytoca* could be isolated significantly more often in the faeces of AS patients when compared to those with osteoporosis or rheumatoid arthritis (62). Two American groups have also found an increased isolation of *Klebsiella* microbes in the faecal cultures from active AS patients whether they were identified by high CRP levels (63) or defined clinically (64). However other groups could not find such an association (65-67) but these conflicting results could be due to different methods used for the collection and culture of the faecal specimens, and whether

the samples were obtained during active or inactive stages of the disease.

(8)   Plasma cells in the gut mucosa which are one of the main source for total serum IgA and especially secretory IgA production were found to be more abundant in English patients with AS than in healthy controls (68)

(9)   Secretory IgA and IgM were both found to be significantly increased in the gut jejunal lavage fluid of AS patients from Sweden when compared with healthy controls (69). This indicates that anti-*Klebsiella* antibodies are being secreted directly into the cavity of the gut, probably to control the quantity of the bowel bacteria.

(10)   A significant association between increased IgA antibodies to *Klebsiella* and gut inflammation was found to occur in Finnish patients suffering from AS (70).

(11)   Swedish AS patients were found to have an increased gut jejunal production of *Klebsiella* antibodies when compared to rheumatoid arthritis patients or controls (71).

(12)   More than three decades ago, in 1969, a French group had found evidence of inflammatory and sclerotic abnormalities in the pelvic and sacral lymph nodes in AS patients using lymphangiography . The lymph nodes changes seemed to precede the development of radiological changes in the lumbar spine and sacroiliac joints (72). The French workers went ahead and speculated that the disease process firstly affected the soft tissues of the lymph nodes before it affected the bony parts of the spine and pelvis. They then suggested this was evidence for a bacterial infective and inflammatory process, a remarkable suggestion made 4 years before the discovery of the link between HLA-B27 and AS and 7 years before the discovery of the link between *Klebsiella* and AS.

The results of these studies support the hypothesis that an important link exists between gut lesions and AS. The main bacterial immune response would appear to involve mucosal immunity with signs of overt or more commonly covert or sub-clinical *Klebsiella* intestinal infections occurring in patients with AS. The bacterial infective *Klebsiella* attack seems to occur in AS patients from many different countries, located in both the Old and New Worlds.

POSSIBLE PATHOGENETIC ROLES OF *KLEBSIELLA* IN "AS" DEVELOPMENT

The world-wide distribution of increased *Klebsiella* antibodies in AS patients would strongly support the hypothesis that exposure to this microbe occurs in this disease. Antigens expressed on the surface of bacterial molecules are more likely to involve humoral immunity.

One of the main targeted antigens of *Klebsiella* antibodies is to capsular lipopolysaccharides (LPS) (73,74) which constitute the main part of the outer membrane wall of these microbes. Thus various antigenic molecules present on the surface of *Klebsiella* microbes including capsular polysaccharides enzymes produced by these microbes such as nitrogenase reductase and pullulanase, may all be involved in triggering the disease development in AS (75). In an ELISA study, English AS patients were found to have significantly elevated levels of IgG and IgA antibodies against *Klebsiella* LPS ($p < 0.001$) and IgA antibodies against *E.coli* LPS ($p < 0.05$) (76). The latter less significant result against *E.coli* could be explained by cross-reactivity between these two enterobacterial microbes.

The cross-reactive protein sequence (QTDRED) is located on the outer rim of the peptide binding cleft of the HLA-B27 molecule and hence more accessible to anti-nitrogenase and anti-pullulanase *Klebsiella* antibodies. The anti-*Klebsiella* antibodies produced in the gut lymphoid tissues may be carried through the local lymphatic plexuses and bind to the cross-reactive antigens present in the nearby joint tissues such as the sacro-iliac joints, lumbar spine, other sites of tendon attachments and even carried via the circulatory pathways to remote tissues such as muscles and the uveal tract (77,78).

The types of cross-reactive antibodies produced following *Klebsiella* infections would determine the anatomical location of the lesions. These are of two types: those reacting with HLA-B27, which may attack the chondrocytes or cartilage cells in the spine and those reacting with type I, III and IV collagens, which are components of spinal tissues. The binding of these *Klebsiella* cross-reactive antibodies, when present in high titres may trigger the onset of inflammatory cascades such as, the complement system as well as various cytokines resulting in the pathological changes with consequent fibrosis and calcification with bony changes, leading to the development of AS (Figure 2).
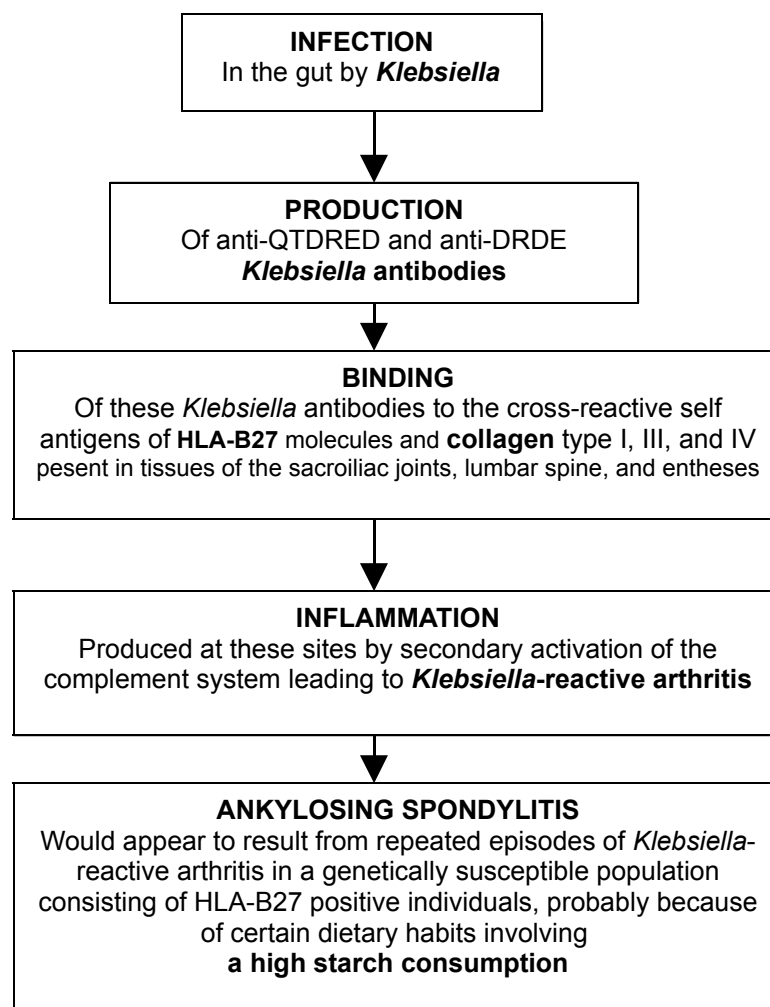
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                    ┌─────────────────────────────┐
                    │         INFECTION           │
                    │   In the gut by Klebsiella   │
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              │            PRODUCTION                │
              │  Of anti-QTDRED and anti-DRDE        │
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     │                     BINDING                           │
     │  Of these Klebsiella antibodies to the cross-reactive │
     │  self antigens of HLA-B27 molecules and collagen      │
     │  type I, III, and IV pesent in tissues of the         │
     │  sacroiliac joints, lumbar spine, and entheses        │
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     │                  INFLAMMATION                         │
     │  Produced at these sites by secondary activation of   │
     │  the complement system leading to                     │
     │  Klebsiella-reactive arthritis                        │
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     │              ANKYLOSING SPONDYLITIS                   │
     │  Would appear to result from repeated episodes of     │
     │  Klebsiella-reactive arthritis in a genetically       │
     │  susceptible population consisting of HLA-B27          │
     │  positive individuals, probably because of certain    │
     │  dietary habits involving                             │
     │  a high starch consumption                            │
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Figure 2: Proposed sequential pathogenetic events in the development of AS.

The extensive evidence reviewed, involving immunogenetic, microbiological and serological studies carried out independently by various groups leads to the suggestion that AS is a form of "*Klebsiella* reactive arthritis." This entity could be defined by objective tests involving three main lines of experimental findings (79):

(1)   *Klebsiella* microbes can be isolated from the bowel of active
         AS patients.

(2)   *Klebsiella* antibodies can be identified in the sera of active
         AS patients.

(3)   *Klebsiella* antigens cross-react with HLA-B27 and collagens
         I, III and IV self-molecules.

One of the main difficulties in accepting the idea of "*Klebsiella* reactive arthritis" is the lack of epidemiological association between *Klebsiella* infections and AS in comparison to other "reactive arthritides" associated with other Gram negative bacteria, such as *Salmonella, Shigella* and *Yersinia.* It is possible that *Klebsiella* may be cleared by the host immune response, after its triggering function, hence removing the chance of isolating the bacteria from the bowel, but the initiated response would still continue to proceed against cross-reactive self-antigens (80). However the concept of "*Klebsiella* reactive arthritis" could be placed in the same category as other types of "reactive arthritis", which are caused by gut or urinary bacteria such as *Yersinia, Shigella, Salmonella, Campylobacter Ureaplasma* and *Chlamydia.* In these conditions the diagnosis can be confirmed by finding elevated specific anti-bacterial antibodies in the sera with or without isolation of the causative microbes from the bowel or urinary tract of these patients (81), a situation that could also occur in AS patients.

One point of criticism concerning the *Klebsiella* involvement in AS, is that *Klebsiella* strains that carry the nitrogenase enzyme are predominantly soil bacteria, expressed only in anaerobic conditions and found only in the faeces of small proportion of AS patients (82). The strain of *Klebsiella* which is implicated in the pathogenesis of AS is *Klebsiella pneumoniae* and *Klebsiella oxytoca,* both of which produce nitrogenase and pullulanase enzymes and their presence in small groups of susceptible individuals could explain why only 2% of the general population are likely to develop AS. If only 20% of HLA-B27 positive individuals develop some symptoms of "B27 disease" we are still dealing with a large population of sufferers who require early and adequate treatment of their painful condition.

Various theoretical models have been proposed to explain the "AS-B27" association (79) such as "one gene" and "two gene theories."

The "one gene" proposal still stands firm, as no other genes apart from HLA-B27 have so far been discovered that show such a remarkable association. The 4 different variants of the "one-gene theory" include plasmid, chemotaxis, receptor and molecular mimicry theories.

(1) Plasmid theory: The Geczy group from Sydney had proposed that rabbit antisera raised against plasmids present in some bacteria, such as *Klebsiella, Salmonella* or *Shigella,* possessed cytotoxic activity against HLA-B27 positive

lymphocytes obtained from AS patients but not against those obtained from healthy HLA-B27 positive controls (89). In a later study, however it was found that anti-*Klebsiella* antibodies raised in rabbits could bind equally HLA-B27 positive lymphocytes from AS patients as well as healthy controls (44).

(2) <u>Chemotaxis theory</u>: The chemotaxis model was originally proposed by a group from Helsinki, in which it was suggested that HLA-B27 positive cells had increased chemotactic activity compared to HLA-B27 negative cells, which will eventually lead to the development of AS (84). Another group has also been able to confirm a specific HLA-B27 linked chemotactic property (85). A logical consequence of this model is that because of the increased chemotactic activities of the immune cells, which all carry HLA-B27 antigens, the non-specific inflammation will be enhanced in response to other viral and bacterial antigens. However it does not appear that HLA-B27 individuals are more likely to suffer from viral or bacterial infections.

(3) <u>Receptor theory</u>: The receptor theory suggests that the HLA-B27 molecule acts as a groove or cavity which binds some external environmental antigen. The model has been extensively investigated by many research groups, but no specific ubiquitous peptide binding to the HLA-B27 groove has been identified (86,87). Although it is generally agreed that most HLA molecules bind peptides of 8 – 12 amino acid residues in length, it has also been reported that HLA-B27 molecules could bind peptides of up to 33 amino acids in length (88). It has also been shown that HLA-B27 monocytes express free heavy chains (89) and can form disulphide bonds (90) but its relevance to AS is unclear.

(4) <u>Molecular mimicry theory</u>: The molecular mimicry is based on the results of many experimental studies and agrees with similar observations made in other diseases such as rheumatic fever and rheumatoid arthritis (91,92). Molecular modelling of the HLA-B27 molecule in the form of disulphide binding through the cysteine residue at position 67 would result in partial unwinding of the alpha-helix in the peptide binding cleft (93) and this together with increased levels of HLA-B27 expression in AS patients (94) could make this molecule more accessible to the cross-reactive anti-*Klebsiella* antibodies.
  The prevalence of IL-10 and the relative frequency of TNF-alpha and interferon-gamma cytokines among patients with "reactive arthritis" (95) and possibly AS, might help the causative microbial agents to persist inside the body, thereby increasing the chances of these microbes to trigger the autoimmune response because of the continuous exposure of the body tissues to high levels of cross-reactive anti-microbial antibodies.
  Biological evidence obtained from several animal models and other diseases such as rheumatic fever and Sydenham's chorea indicates that molecular mimicry is more than an accidental observation (96).
  Furthermore, several other diseases such as pemphigus, type I diabetes, primary biliary cirrhosis, Crohn's Disease, and Multiple Sclerosis could also be the end result of a molecular mimicry mechanism (80).

IMPORTANCE FOR EARLY DIAGNOSIS IN THE MANAGEMENT OF PATIENTS WITH "AS"

Several years are required for the classical clinical features of AS, particularly radiological evidence of sacroiliitis to appear before the diagnosis can be established. By the time the disease is diagnosed, the destructive pathological events have occurred and at this late stage it appears to be difficult to reverse the damaging effects of the disease.

Nothing upsets patients with "B27 disease" more, than to be told, "You cannot have AS because you do not have X-ray changes of sacroiliitis."

Early diagnosis is clearly required but the problem is that "B27 disease" has only been recognised over the last 30 years since the discovery of the link between AS and HLA-B27.

The association of inflammatory back pain together with certain other features such as inflammation at the tendon enthesis insertions, knee, hip, shoulder, rib cage and ankle arthritis and acute anterior uveitis, as well as the presence of HLA-B27 greatly increases the probability that the condition should be considered as "AS" (97).

In a 10-year follow-up study of probable AS, it was observed that the progression from early, incomplete AS to definite AS as shown by radiological sacroiliitis would occur after 9 +/- 6 years (98).

In Lewis rats with *Yersinia* triggered arthritis, it was observed that the development of arthritis was completely prevented within the first few days of antibiotic treatment, whilst if treatment was delayed until a week or two later when the rats had developed arthritis, no improvement in the joint lesions was observed (99).

This might be comparable to human diseases with a similar pathology and explain the successful effect of ciprofloxacin on patients with *Yersinia* reactive arthritis in whom the mean disease was relatively short (1.9 years) (100), and its failure in patients with longer disease duration (4.9 years) (101).

More than 20 years ago, in 1980, Agarwal from Pittsburgh has emphasized the need for early diagnosis of AS and suggested that patients showing clinical features of the disease but no evidence of radiological sacroiliitis should be diagnosed as having "pre-ankylosing spondylitis" or pre-AS for short (102).

"AS" could be considered as the end result of repeated attacks of *Klebsiella* reactive arthritis.
 It is proposed that 2 types of *Klebsiella* reactive arthritis could be recognized under the terms of this definition:

"Early *Klebsiella* reactive arthritis" and "Advanced *Klebsiella* reactive arthritis."

The taxonomic or diagnostic features of these 2 conditions can be defined as follows:

<u>Early *Klebsiella* reactive arthritis or pre-</u>AS is said to be present in an individual, who has:

(1)   Arthritic symptoms in the spine and large joints (hips,knees,ankles) for at least 3 months, associated with "early morning stiffness" which is relieved by exercise.

(2)   Presence of tendon insertion inflammation at the entheses or uveitis with or without a positive family history.

(3)   Presence of HLA-B27.

(4)   Presence of elevated anti-*Klebsiella* antibodies (Titre> 95% confidence limit above healthy controls) during episodes of inflammation, with or without *Klebsiella* isolation in the stools.

(5)   No radiological evidence of sacroiliitis.

<u>Advanced *Klebsiella* reactive arthritis or classical AS</u> is said to be present in an individual who has all the features of "Pre-AS" (conditions 1-4) but also has radiological evidence of sacroiliitis.

Thus "Advanced or Late *Klebsiella* reactive arthritis" would correspond to the classical definition of "AS."

Active disease in such patients can be accurately defined by using the "Bath AS Disease Activity Index" (BASDAI), especially when used in conjunction with increased levels of "erythrocyte sedimentation rates" (ESR's) and "C-reactive protein" (CRP) measurements (103).

The use of the above proposed criteria for the diagnosis of pre-AS should be carried out only after a rheumatologist has excluded other rheumatic diseases.

There still remains a small group of "classical AS" patients who are HLA-B27 negative and who fit the criteria defined by the Linssen group (104). These could be called "HLA-B27 negative AS." Whether *Klebsiella* is involved in this group is at the moment unclear. Also Reiter's disease patients often in time develop features of AS.

A considerable number of patients with arthritis affecting several joints, unlike those with reactive arthritis, are lacking the usual evidence of an antecedent infection. Some of these patients may in fact be cases of pre-AS or reactive arthritis following infection by *Klebsiella* or other Gram negative bacteria. In reactive arthritis the identification of the initiating bacterial agents is not always possible because of the relatively long intervals weeks to months, between the onset of infection and development of arthritic features. Moreover, the triggering infection may be asymptomatic and in a considerable number of cases no inciting microbes can be found. Such a situation may also occur in patients with early AS or even in patients with Reiter's syndrome who then go on to develop classical AS.

Based on the proposed criteria, early diagnosis of pre-AS or early *Klebsiella* reactive arthritis is possible and therefore specific therapy can be started during the early stages of the disease.

The incidence of theumatic fever, an autoimmune disease caused by *Streptococcal* infections of the upper respiratory tract, has almost disappeared in the Western World because of the early use of antibiotics. However, in the Third world where access to antibiotics is difficult, it still remains a serious problem. Based on such observations in the eradication of rheumatic fever, a similar approach could be used in the early treatment of AS.

PROPOSAL FOR AN ANTI-MICROBIAL THERAPY IN PATIENTS WITH "AS"

Currently the management of patients with AS includes two main approaches:

(A) The first one involves the use of "non-steroidal anti-inflammatory drugs" (NSAIDs) and sometimes "disease modifying anti-rheumatic drugs" (DMARDs) (105) or even the use of immunosuppressive and biologic anti-tumour necrosis factor (anti-TNF) therapies. (106-108)

(B) The second one involves physiotherapy, spa exercises and postural education (109) to prevent stiffness and deformities.

The NSAIDs and DMARDs and especially the immunosuppressive and biologic agents are effective in alleviating pain, reducing inflammation and improving the quality of life when combined with exercises to maintain physical function (110).

However these drugs cannot reverse the existing spinal lesions and are associated with deleterious side effects, such as an increased risk of serious bacterial infections (111) or even life threatening histoplamosis (112).

The use of other therapeutic measures together with NSAIDs and DMARDs to eliminate or reduce the *Klebsiella* microbes could therefore have beneficial effects on the patients with this disease.

These new therapeutic strategies would involve 2 main lines:

(1) Antibiotic treatment

Sulphasalazine was and is still considered by many independent groups as one of the most effective and well tolerated drug in the treatment of patients with AS. A 26-week, placebo control trial showed that enteric coated sulphasalazine seemed to be effective and well tolerated in AS patients (113). In a 3 year placebo controlled trial of sulphasalazine, a reduced frequency of peripheral arthritis was observed in the treated but not in the control group of AS patients (114). A 6 month randomised, multi centre, double blind, placebo controlled trial of sulphasalzine in AS patients resistant to treatment with NSAIDs showed that sulphasalazine had greater efficiency than placebo (115). Norwegian AS patients treated with sulphasalazine, showed a significant decrease in total IgA and secretory IgA in the jejunal perfusion fluid when compared to healthy controls (69). A significant decrease in the concentration of *Klebsiella* antibodies during the 26 weeks of sulphasalazine treatment has been

reported in Finnish AS patients when compared to controls (116). Sulphasalazine was also found to be beneficial in preventing Recurrences and reducing the severity of uveitis associated with AS (117). Many groups have shown that the anti-microbial component sulphapyridine is the active moiety of sulphasalazine (118-120).

Hence the beneficial effects of sulphasalazine could possibly result from the anti-microbial action and this may further strengthen the evidence of a role for microbes in the pathogenesis of AS. Nevertheless, it should be emphasized that AS patients with peripheral arthritis show the beneficial effects of sulphasalazine more readily than those with axial involvement. Although thalidomide is suggested to have immunomodulatory rather than anti-microbial properties, its beneficial effect on more than 50% of AS patients, is an encouraging observation (121).

Ciprofloxacine was found to be effective in the early tratment of *Yersinia* reactive arthritis. *Yersinia* microbes were found to be eliminated from the gut associated lymphoid tissues in 6 of 7 patients receiving ciprofloxacin compared with none of 9 patients receiving placebo. Furthermore patients receiving placebo had higher levels of circulating IgA antibodies against *Yersinia* than patients treated with ciprofloxacin (100). More recently it has been observed that long term treatment with lymecycline in patients with acute reactive arthritis, had decreased the duration of arthritis in those with *C.trachomatis* triggered reactive arthritis (122).

Certain antibiotics have been tested and found to be effective against *Klebsiella* infections. Some of these antibiotics include: Cephalosporins, aminoglycosides, mezlocillin, piperacillin, ciprofloxacin, aztreonam, trimethoprin-sulfamethoxazole and imipenem (123). However a search for finding other antibiotics which might have more specific and effective actions on *Klebsiella* microbes is clearly necessary.

As yet there is no clinical data to support the beneficial use of antibiotics in AS (124) but a substantial obstacle is the difficulty of identifying early cases (125) when such therapy might abort the disease process.

(2) Low Starch Diet

Dietary starch (BREAD,POTATOES,CAKES and PASTA) provides nutrient materials necessary for the growth of gut bacteria.

Normal subjects fail to absorb 5-20% of dietary starch present in wheat flour, commonly present in bread and pasta, as assessed by post meal oral hydrogen excretion, thereby allowing the residual carbohydrate compounds to be used as substrates for the growth of colonic bacterial microbes (126).

In vitro studies have shown that the mean number of *Klebsiella* microbes for 3 different sugars (glucose, sucrose and lactose) per gram of substrate was found to be significantly higher when compared to the value obtained following incubation with 11 different amino acids, thus showing that protein components are relatively inefficient substrates for bacterial growth and proliferation (127).

Furthermore, *Klebsiella* microbes do not seem to grow on plant and fruit cellulose (128).

In an experimental study, rats that had been fed potato starch showed an increase in the numbers of gut bacteria (129).

In a clinical study, Finegold and coworkers carried out bacterial cultures on 47 vegetarian subjects on a high starch/low protein diet and compared these to 45 American subjects on an omnivorous diet involving low starch and high protein consuption. The mean number of *Klebsiella* microbes in the "high starch" group was 30,000 bacteria per gram of faeces compared to a value of 700 bacteria per gram of faeces in those on a high protein diet (130).

In another study, it was observed that the majority of AS patients, who were on the "London Low Starch Diet" (NO BREAD, NO POTATOES, NO CAKES, NO PASTA and NO RICE), a diet low in starch and high in proteins and fruits, claimed a drop in the severity of their symptoms as well as a reduction in their requirements for NSAIDs. This clinical improvement was found to correlate with a decrease in total serum IgA, which measures gut flora and also a decrease in inflammation as measured by ESR (131).

A group of 74 randomly chosen AS patients attending the "AS Research Clinic" at the Middlesex Hospital in London were asked to participate in an open study, involving a "LOW STARCH, HIGH PROTEIN, HIGH VEGETABLE AND FRUIT DIET" (Table 3) over a period of 10 months. The patients were being treated with phenylbutazone, indomethacin or other NSAIDs. A comparison of haemoglobin and ESR was carried out before and after the completion of the trial. The mean ESR came down significantly in all the haemoglobin groups studied with the highest drops in ESR measurements in those with low hemoglobin levels. Thus the use of a "low starch diet" could be helpful in preventing the growth of the gut *Klebsiella* bacteria, thereby reducing inflammation in these AS patients and producing clinical improvement (132).

It is suggested, therefore, that a combination of *Klebsiella* specific antibiotics given in short courses, especially during attacks of *"Klebsiella* reactive arthritis" together with a "low starch/high protein-high fruit diet" may have a beneficial effect on AS patients especially if started during the early stages of the disease. These measures should be in addition to existing methods of treatment, such as NSAIDs and immunosuppressive and biologic agents. The use of the "low starch/high protein-high fruit diet" may also reduce the need for high doses of NSAIDs and immunosuppressive and biologic agents thereby decreasing the likelihood of undesirable side effects. Prospective controlled studies are required to determine the relevance of these measures in the treatment of patients suffering from AS.

Table 3: London "AS Low Starch Diet;" Recommended treatment for AS patients

Decreased intake of the following starch containing foods

(1) **Bread and Biscuits**
- Bread (white, brown, wholemeal, etc) in very limited amounts.
- Crispbreads, biscuits ('cookies'), cream crackers, and twiglets.
- Cakes, puddings, and pies.
- Popcorn.

(2) **Pasta**
- Macaronis, noodles, spaghetti, pastry, pizzas, and other pastas.

(3) **Rice**
- Brown, white, boiled, fried, or in puddings.

(4) **Potatoes**
- Baked, boiled, fried, roasted, or mashed potatoes and potato crisps ('chips').

Increased intake of proteins, vegetables, and fruits

(1) **Meat**
- Beef, pork, lamb, bacon, salami, corned beef, luncheon meat, potted meat, ham, and veal.
- Chicken, turkey, duck, or any other poultry meat.

(2) **Fish**
- White fish such as cod, haddock, plaice, sole, etc.
- Shellfish such as crab, lobster, prawns, scampi, cockles, mussels, and oysters.
- Herring, salmon, mackerel, tuna, sardine (tinned in oil, brine, or tomato).

(3) **Milk and Milk Products**
- Fresh, dried or condensed milk, plain and flavored yoghurts, all types of cheese.

(4) **Eggs**
- Prepared in any manner.

(5) **Vegetables**
- All green vegetables such as cabbage, sprouts, courgettes, spinach, broccoli, and carrots, cauliflower, mushrooms, peppers, etc.
- All salad vegetables like lettuce, cucumber, celery, tomatoes, watercress, etc

(6) **Fruits**
- All kinds of fruits.

CONCLUSIONS

(1) *Klebsiella* microbes have a direct role in the pathogenesis of AS based on the results of microbiological, serological and molecular studies carried out by different independent research groups throughout the world.

(2) AS would appear to be the end result of recurrent episodes of *Klebsiella* reactive arthritis.

(3) Patients with pre-AS can be identified by using the new diagnostic criteria and can be treated with anti-*Klebsiella* therapy, involving "low starch/high protein-high fruit diet" together with existing methods of treatment, which could possibly reduce the severity and the number of relapses in this disease.

(4) Prospective controlled studies are required to evaluate anti-*Klebsiella* in the management of AS.

ACKNOWLEDGEMENTS

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