E. Wilkes, E. S. Meek

Rheumatoid Arthritis:
Review of Searches for an Infectious Cause. Part I

Summary: No distinctive pattern has yet emerged from the accumulated mass of results that would provide a generally acceptable hypothesis of the etiology of rheumatoid arthritis. A number of immunologic aberrations have been described, but there has been no identification of a key immunologic defect that might link together the various components of the immune response into an agreed pattern. The possibility of a persistent antigenic stimulus arising from an infection cannot be confirmed or refuted. If a virus is involved, it would seem more likely to be a "slow" virus rather than a commonly recognized form, but there is no strong candidate of this type in view. Despite the fact that mycoplasmas are undoubtedly arthritogenic in other species, their role as an etiologic agent in rheumatoid arthritis has not been proven. The idea that bacterial cell wall peptidoglycan may provide a persistent stimulus has much to offer, but it is not possible at this stage to accept peptidoglycan as a recognized etiologic factor. This suggestion will, however, undoubtedly stimulate much further investigation.

Zusammenfassung: Rheumatoide Arthritis: Forschungsübersicht zur Infektions-Ätiologie. Bisher hat sich aus der Masse von angesammelten Befunden kein spezifisches Muster ergeben, das als allgemein gültige Hypothese der Ätiologie der Rheumatoiden Arthritis dienen könnte. Eine Anzahl immunologischer Anomalien wurde beschrieben, ohne daß ein immunologischer Schlüsseldefekt hätte erkannt werden können, der die verschiedenen Anteile der Immunantwort zu einem passenden Muster zusammenfügen könnte. Die Möglichkeit eines anhaltenden, von einer Infektion ausgehenden Antigen-Stimulus kann weder bestätigt noch widerlegt werden. Falls ein Virus beteiligt ist, dürfte es wahrscheinlicher ein „slow virus" sein als eine allgemein bekannte Form, doch ist bisher kein ernsthafter Vertreter dieses Typs in Aussicht. Trotz der Tatsache, daß Mykoplasmen zweifellos bei anderen Species arthritogen wirken, ist ihre Rolle als ätiologisches Agens der Rheumatoiden Arthritis nicht belegt worden. Die Vorstellung, daß das Peptidoglykan der Bakterienzellwand einen Dauerstimulus darstellen könnte, hat viel für sich, aber bei dem jetzigen Stand der Dinge ist es nicht möglich, das Peptidoglykan als einen gesicherten ätiologischen Faktor anzuerkennen. Diese Vermutung wird jedoch zweifellos viel Anregung für weitere Forschungen bringen.

Introduction

In discussing the etiology of rheumatoid arthritis, *Hirschhorn* (1) likened the situation to that of "the blind men and the rheumatoid elephant."

The description of rheumatoid arthritis (RA) as an autoimmune disease emphasizes the immunologic abnormalities that are a prominent feature of the disorder, but the initiating factor has yet to be identified. It remains to be seen if this is an exogenous infective agent, and all efforts to substantiate the view that RA is of infectious origin have failed so far.

In this review, the immunologic phenomena will be discussed briefly since these may represent the response to an underlying infection. The possible involvement of viruses, mycoplasmas, and bacteria will then be considered, citing some representative publications in order to present a general overview of the extent of searches for an infectious agent.

Hereditary Susceptibility

Although RA tends to develop in more than one member of an affected family, there is no simple Mendelian pattern of inheritance; however, a genetic predisposition is indicated.

Studies of histocompatibility genes have shown a strong correlation between type HLA-B.27 and ankylosing spondylitis (2, 3), also between this antigenic type and Reiter's disease (3, 4). This particular haplotype has not been implicated in RA, but recent work has revealed a significant degree of association between HLA-DW4 and RA (5). The demonstration of a heritable predisposition does not, of course, exclude the possible existence of a second factor, that could be an infectious agent, as an essential component of the etiology. *Zabriskie* (6) described a serologic reaction between several HLA typing antisera and group A streptococcal membranes, and suggested that a histocompatibility antigen may share an antigenic determinant with a putative causative microorganism.

Immunologic Reactions

In the synovial fluids of RA patients there is decreased activity and decreased concentration of various complement components, indicative of the consumption of complement in the disease process. Material resembling immune complexes has been detected in the joint fluids from both seropositive and seronegative cases (7), showing again that the complement system has been activated. Both the classic and the alternative pathways of complement activation appear to be involved (7, 8, 9, 10), and study of the breakdown products implicates both pathways in 93% of seropositive specimens, and in 89% of seronegative samples (7). A striking feature of RA is the

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E. Wilkes, B. Sc. Dr. *E. S. Meek,* Department of Pathology, College of Medicine, University of Iowa, Iowa City, Iowa 52242, USA.
Reprints: Dr. *E. S. Meek*

extent of activity directed against immunoglobulins. Auto-immunoglobulins can be classified as either: a) those that react with the Fc determinants of the IgG molecule, of b) those that react with Fab or F(ab')₂ determinants which are accessible only after digestion of IgG molecules by proteolytic enzymes (11). The first group (a) are the rheumatoid factors (RF), and the second (b) are serum agglutinators, mostly IgG molecules. Both appear to be present in RA.

In most cases, the anti-immunoglobulin antibodies are rheumatoid factors (RF), and these are found in serum, joint fluid, and in the inflammatory tissues. Rheumatoid factors reacting with the Fc fragment of IgG exhibit a wide spectrum of specificities, and include sites in both the CH2 and CH3 domains of the Fc fragment (12). Other types of anti-IgG antibodies have been detected in the synovial fluid of RA patients, and may form part of immune complexes (13, 14, 15). In addition, antibodies directed against IgM, IgA, IgG, and IgE, together with some rare anti-IgG antibodies directed against the Fab region, have been detected but their significance in RA is not known (16).

The anti-immunoglobulins in serum, synovial fluid, and tissues, are themselves nearly always of the IgM or IgG class (IgM-RF; IgG-RF), although *Kunkel* (17) described IgA-RF also. The RF that bind to autologous IgG in joint fluid and joint tissues fix complement, but in contrast the circulating RF does not bind complement.

There is an inverse relationship between the activity of complement factors and the amount of IgG immune complexes in synovial fluid in actively inflamed joints (18). A substantial amount of IgG-RF is present in these complexes, and apparently similar complexes containing IgG and complement factors are found in the macrophages that are present in the synovial fluid (19). Large amounts of immune complexes are also present in the surface layers of the synovium, both within cells and extracellularly, in both seropositive and seronegative RA, and also in juvenile RA (JRA) (16, 20, 21).

There seems to be no significant difference between the histologic findings in seropositive and seronegative cases, and on this basis it has been suggested that the serum IgM-RF contributes little to the pathogenesis of the joint lesions since the distinction between positive and negative refers to the presence or absence of IgM-RF (22).

The reason for the reaction against self-immunoglobulins remains unknown.

One possibility is suggested by the preliminary report of a configurational difference between normal and rheumatoid IgG that was particularly marked in the neighborhood of the hinge region of the F(ab')₂ fragment (11). Such a-difference may incite the formation of antibodies against the aberrant IgG molecule; this work needs to be confirmed.

Not only is there a humoral antibody reaction against immunoglobulin, but cell-mediated immunity (CMI) a-gainst IgG is also provoked (23, 24, 25). CMI against

synovial membrane antigens is present in almost all patients with RA, but whether the antigenic stimulus refers to an altered cell component, or to a pathogenic agent associated with the cell membrane, has yet to be determined (23); IgG, which is present on the synovial membrane, may serve as a surface-associated antigen.

Although plasma cells and their B-lymphocyte precursors are responsible for anti-immunoglobulin activity, an inflammatory reaction can occur in RA patients with B-cell deficiency without abundant immune complexes; this may result from activation of the alternative complement pathway (26). Also, a CMI reaction may stem from the presence of T-cells and of Fc-bearing lymphocytic cells that interact with IgG and are present in RA synovial fluid and serum.

There is some evidence of a functional defect in CMI in patients with RA (27, 28, 29), but the relationship between humoral antibody changes and cellular factors is not clear. Antibody-dependent cell-mediated cytotoxicity (ADCC) is a function that depends on the integrated activity of cells and humoral antibody. Unsensitized lymphocytes carrying an Fc receptor are specifically activated by exposure to an exogenous antigen ("effector" cells) and their cytotoxic response is measured against target cells that have been sensitized by exposure to IgG antibody directed against the exogenous antigen. There is evidence of impaired function of lymphocytes from patients with RA when measured in terms of this response; sera containing RF depress the response by reacting with both the effector cells and target cells, suggesting that the impaired performance of the RA lymphocytes is due to a humoral inhibitory factor rather than an intrinsic defect of the lymphocytes.

If some form of immunodeficiency underlies the development of RA, this may be manifest by failure to eliminate an infective agent, so leading to the establishment of a persistent infection.

The deposition of immune complexes in tissues is associated with the preceding persistence of soluble antigen-antibody complexes in the circulation. Soluble complexes, that contain an excess of antigen over antibody, may form as the result of production of antibody with low affinity for the antigen and in these circumstances, the antigen would not be eliminated (30). Production of low-affinity antibody may reflect a genetic predisposition. It has been shown that some microorganisms can induce the formation of antibody that reacts not only with the microorganism but that cross-reacts with a similar normal host antigen. The commonality of antigenic determinants in β-hemolytic streptococcus cell membranes, myocardial sarcolemmal fibers, and smooth muscle fibers, has been described (31, 32).

Infectious Agents

Although it has long been suspected that rheumatoid arthritis (RA) is of infectious cause, there is still no evidence to substantiate this view.

Viruses, mycoplasmas, diphtheroids, and L-forms of bacteria have been considered as potential candidates, but it is not possible to identify a microorganism as a causal agent even in these general terms.

Attempts have been made to transmit some form of arthritis from patients with RA to experimental animals. Although the induction of arthritis in chicks and mice that received cell-free extracts of synovial tissue from RA patients has been reported (33, 34, 35), efforts to confirm these results have not been successful (36, 37). The possibility that household pets might serve as a reservoir for an etiologic agent responsible for RA has been suggested by *Gottlieb* et al. (38) who found that during the five-year period prior to the onset of disease, there was a greater prevalence of pets in the households of 105 patients with RA than in those of the control groups.

Viruses

Many different viral infections are accompanied by arthritis. These include vaccinia, smallpox, rubella, measles, influenza, mumps, viral hepatitis type B, varicella, infectious mononucleosis, and illnesses caused by various enteroviruses, adenoviruses and togaviruses. The arthritis induced by these agents is, however, transient. Proof that a virus can cause chronic recurrent synovitis in man is lacking.

Rubella virus has received much attention as a potential candidate since both the wild and vaccine strains of virus are known to cause a transient arthritis in humans, and there is some experimental evidence suggesting that it can induce a chronic arthritis in rabbits (39, 40, 41, 42). Although a follow-up study of a small series of human rubella infections showed no evidence for the development of RA (43), the results of one investigation did indicate a selective depression of cell-mediated immunity against the virus in those who developed arthritis after rubella immunization (44). Increased levels of rubella serum antibody have been reported in patients with juvenile rheumatoid arthritis (JRA) (45, 48), but these results were not supported by those of other investigators (44, 46, 47). Rubella viral antigen has been reportedly detected, however, in the synovial fluid of some JRA patients (48). In cases of RA, rubella serum antibody levels are not significantly different from those of controls (47, 49, 50, 52), and viral antigen could not be detected in RA synovial fibroblasts (49).

Serologic studies of other viruses have uncovered no significant association between RA and the titers of specific antibody against cytomegalovirus (51, 52); herpes simplex (52); varicella-zoster (52); Epstein-Barr virus (47, 52, 53); influenza A (50); parainfluenza type 1 (53); mumps (48, 52); poliovirus (48); or that of hepatitis type B (52, 54). Although raised levels of antibody to measles virus have been reported in RA synovial fluid (50), serum levels of this antibody do not differ from those of controls (48, 52, 53).

Numerous attempts have been made to isolate a virus from joint tissue and fluids, or to detect virus-specific products, but these efforts have been unrewarding despite the use of a variety of sophisticated technics (52, 55, 56, 57).

Electron microscopy of RA synovial tissue has also failed to provide firm morphologic evidence for the presence of a virus. Some investigations have revealed structures that may be of viral origin but their identity is uncertain (58, 59, 60, 61, 62, 63, 64), and an ultrastructural examination of ten lines of cultured rheumatoid synovial cells showed no components resembling viruses (65).

The possibility of a "slow" viral infection has been suggested. An example of this type of infection is subacute sclerosing panencephalitis, caused by measles virus and characterized by an insidious onset and slow progression. "Slow" viruses are unusually difficult to isolate and there is no evidence so far for their involvement in RA.

It can be argued that failure to isolate a virus may merely reflect the lack of an appropriate system for culture in vitro, and failure to see viral specific material by electron microscopy may result from the absence of a morphologically discrete structure. Although the possibility that RA is caused by a virus cannot be excluded, so far there has been no convincing evidence in favor of this view.

Mycoplasmas

Mycoplasmas are distinguished from other bacteria by their hereditary inability to form a cell wall. These microorganisms have been shown to cause various forms of arthritis in several species of animals and birds in both natural and experimental conditions.

Arthritis develops in swine after injection with *Mycoplasma hyosynoviae* and *Mycoplasma hyorhinus* (65), in chickens with *Mycoplasma synoviae* and *Mycoplasma gallisepticum* (66), in mice with *Mycoplasma arthritis* (67) and *Mycoplasma pulmonis* (78, 69), in rats with *Mycoplasma arthritidis* (70), in goats with *Mycoplasma arginini* (71), in sheep with *Mycoplasma agalatiae* (72), and in cattle with *Mycoplasma bovigenitalium* and *Mycoplasma mycoides* (73, 74). *Brown* et al. (75) isolated a mycoplasma from the throat of a gorilla with RA, and also from cultures of its wrist synovial tissue. They showed that the mycoplasma from the throat was serologically related to *Mycoplasma salivarium* (a human commensal) and reported that administration of tetracycline resulted in marked improvement of the arthritic condition.

Evaluation of some of the earlier reports on human material is difficult because recovery of mycoplasma was based on the use of cell cultures. The frequency of contamination of cell cultures by mycoplasma, the presence of these microorganisms in some samples of serum, and the difficulties of excluding the presence of mycoplasmas in established cell cultures prior to their inoculation with clinical specimens, leave the validity of some reports open to question. Many of these reports were evaluated by *Mor-*

ton (76), and the problem of contamination of cultures by mycoplasma has also been discussed by *Freundt* (74).

Although some investigators have reported the isolation of mycoplasmas from rheumatoid specimens (77, 78, 79, 80, 81, 82, 83, 84), other investigators have failed to recover these microorganisms or to detect mycoplasmal components (51, 77, 85, 86, 87, 88, 89). Failure to isolate mycoplasma may be due, however, to the mycoplasmacidal action of normal tissue extracts (90). Immunologic studies have also yielded conflicting results. Some have found serologic evidence favoring a significant association of mycoplasmas with RA (79, 91, 92) while others failed to support the findings (93, 94, 95, 96, 97).

The response of RA to treatment with gold salts, and the growth-inhibitory effect of gold on mycoplasmas in vitro, has been proffered as indirect evidence favoring a causal role for mycoplasmas in RA but the failure of chemotherapy to resolve suspected mycoplasma infections does not necessarily prove absence of this microorganism. *Stalheim* (8) administered penicillin, streptomycin, tetracycline, and tylosan, without achieving clinical improvement in calves during an outbreak of mycoplasmal arthritis and pneumonia in which *M. agalactiae* subsp. *bovis* was isolated from the joints, lungs, and trachea.

In all, there is no proof so far that mycoplasmal infection is significantly associated with RA, and the specter of inadvertent contamination looms large in the interpretation of positive findings.

Book Reviews / Buchbesprechungen

J. Drews, G. Högenauer (Ed.):

R-Factors: Their Properties and Possible Control

Topics in Infectious Diseases, Vol. 2

Symposium, Baden near Vienna, April 27–29, 1977
362 pages, 75 figures

Springer Verlag, Vienna, 1977
Price: DM 59.—

Interest in R-factors has been continuous since their discovery in 1953. In spite of the fact that great advances have been made in their molecular biology, it has not yet been possible to limit their importance for chemotherapy. The justification for this further symposium on R-factors is that it was concerned with subjects relevant to clinical epidemiology. Thus in the first part the incidence and ecology of R-factors in different environments and molecular factors responsible for the spread of R-factors are both dealt with. In the next section phenotypic effects of R-factor genes and the metabolism of R-factor DNA are discussed. The concluding round table discussion attempts to define measures against the spread of R-factors, under four main headings: What are the R-factors which make a reservoir of resistant bacteria? What is the size of this reservoir? How is it distributed? What measures could be taken to reduce its size?

Finally, suggestions are made as to the possible pharmaceutical approaches to the control of R-factors, for example the development of antigene transfer agents, the "curing" of bacterial populations of their plasmids, and the replacement of a hospital's resistant bacterial flora with a sensitive population. In addition, there is discussion on whether agents specifically effective against R+ strains could also be useful, as well as those which inhibit either the expression or the function of resistance mechanisms. There was speculation that there might be substrates which, having been modified by an R+ cell, would then kill it.

This symposium is distinguished from numerous others by the close connection achieved between the results of basic research and practical needs. In a well-balanced selection of topics, there is both exact and well presented experimental data and stimulating speculation, especially in the discussion. This book is particularly useful in that it not only provides a full picture of the current situation, but also gives interesting, and sometimes amusing, pointers to possible future developments.

A. Bauernfeind
Max von Pettenkofer University Institute
for Hygiene and Medical Microbiology
Munich

R. E. Trotman

Technological Aids to Microbiology

Edward Arnold Ltd., London, 1978
Preis: £ 4,95

Die Mikrobiologie bzw. das mikrobiologische Labor ist, verglichen mit anderen klinischen Laboratorien, ein Stiefkind, was die Entwicklung technischer Hilfsmittel betrifft.

Daher ist es um so erfreulicher, daß mit dem vorliegenden Buch der Versuch unternommen wird, einen Überblick der z. Z. bestehenden technischen Hilfsmittel und ihrer Möglichkeiten zu geben. Der Autor nimmt nicht für sich in Anspruch, ein komplettes Verzeichnis vorzulegen — eher vermittelt er dem Leser Anregungen für fortschrittlicheres Arbeiten im mikrobiologischen Labor.

Das Buch ist in vier Kapitel aufgeteilt. Nach jedem Kapitel findet sich eine Liste von Literaturstellen für diejenigen, die sich eingehender mit der Materie befassen wollen. Für den interessierten Leser ist sicher auch anregend, zugleich etwas über die historische Entwicklung bestehender technischer Hilfsmittel und Apparate zu erfahren, sowie mit der Problematik „Bakterien der Technik unterwerfen zu wollen" konfrontiert zu werden.

Im 1. Kapitel werden Möglichkeiten der Pipettierung und Reihenverdünnung von Mikromengen infektiöser oder steriler Flüssigkeiten besprochen, dabei auf Vorteile gegenüber Makromethoden hingewiesen sowie auf Fehlerquellen und Genauigkeitsangaben eingegangen.

Das 2. Kapitel gibt einen Abriß verschiedener Bakterienzählmethoden, wobei diese als Kolonie oder durch photometrische Messung erfaßt werden. Die Schwierigkeiten der Zählung von Bakterien mit dem Coulter Counter werden ebenso diskutiert.

Kapitel 3 zählt eigentlich als Erweiterung zu Kapitel 1 und geht auf Methoden der Pipettierung und Überpipettierung chemisch „sauberer" Flüssigkeiten mit Hilfe von Ösen, halb- und ganzautomatischen Pipetten und aufwendigeren Systemen ein.

Kapitel 4 schließlich stellt technische Hilfsmittel vor, mit denen feste Nährböden schneller mit Bakterien beimpt werden können.

Die Gliederung des Buches ist übersichtlich. Es enthält viele gute Abbildungen und graphische Darstellungen. Es stellt einen wertvollen Ratgeber und Informationsquelle dar und sollte als Einzelexemplar in jedem mikrobiologischen Labor stehen.

G. Roos
Universitätskinderklinik
München