

Medical College of Virginia
Virginia Commonwealth University

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Dr. Paul K. Pybus
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The Rheumatoid Disease Foundation
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Dear Dr. Pybus:

I was sorry to hear about your accident. I hope that you are feeling better now that you are home. This letter will recap our conversation of yesterday.

First, with regard to the apparatus I have been using for the thermotropic isolation of amebae, we have proof that the presence of the brass ring does not adversely effect the growth of pathogenic, free-living amebae. I did not think of it at the time you raised the question, but when we did the initial studies demonstrating that amebae could migrate through a 5 micron filter, we also successfully cultivated the amebae that migrated to the warm side (a test done to check our capacity to run the procedure under sterile conditions). Furthermore, we have completed an experiment which entirely lays the speculation to rest. Cultures of amebae (*Naegleria fowleri*, *Naegleria gruberi*, and *Acanthamoeba polyphaga*) were established in petri dishes in the presence or absence of the brass rings used in the device. Growth of amebae was not inhibited in the cultures in which the brass rings were immersed. Thus, leaching of potentially poisonous copper is not occurring. This is as I expected since brass is highly corrosion resistant, and an alloy such as brass is not simply a mixture of the component metals. Also recall that by immuno-histologic techniques (e.g., fluorescent antibody staining) amebae cannot be demonstrated in synovium. These tissues are never placed in the thermotropic isolation unit, nor would they be affected by copper. For future use we will replace the brass with stainless steel (e.g., in testing tumor tissue), though I think the evidence is unequivocal that the brass rings in no way contributed to negative findings in our attempts to identify and isolate an amebal pathogen.

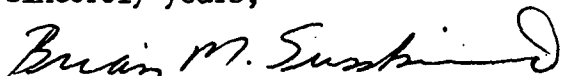
That is not all of the good news. You will recall that after your visit I was headed to Toronto for the Sixth International Congress on Immunology. These conferences are set up like a smorgasbord, and although rheumatoid disease was only one of many entrees, over the course of five days I had the chance to hear presentations by over fifty scientists on the immunology of rheumatoid disease. One group from France presented data that I thought you might find particularly exciting (abstract enclosed). They find a strong correlation between substance P, a neuropeptide, and joint inflammation in rheumatoid arthritis. Substance P affects lymphocytes in ways which would then influence the inflammatory response. I

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immediately thought of your hypothesis that peripheral afferent and sympathetic nerves contribute to the clinical manifestations of rheumatoid arthritis, which you tried hard to persuade me of during your visit. It does lend credence. I still feel we need to focus on the immunologically-mediated mechanisms by which clotrimazole and metronidazole ameliorate rheumatoid arthritis (e.g., affects of the drugs on the abnormal immunologic response in the joint, since the lymphocytes and macrophages are clearly involved in the pathology), but in our protocols we can measure substance P levels and thus see if these data provide further support for the hypothesis.

Also, I have enlisted three rheumatologists here at MCV as consultants for the studies I outlined on the immunoregulatory properties of clotrimazole and metronidazole during your visit. Dick Franson and I will be working more closely as well, and the five of us will meet every four to six weeks to discuss results and to "brainstorm." I will keep you informed of our progress.

Sincerely yours,



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ENCLOSURE: (1 abstract)

cc: Perry A. Chapdelaine, Sr.

3.44.37

EXPRESSION OF THE C3d/EPSTEIN-BARR VIRUS RECEPTOR (CR2/EBVR) ON LYMPHOBLASTOID CELL LINES (LCL) FROM RHEUMATOID ARTHRITIS (RA) PATIENTS. Alice Kahan^(*), Christiane Charriaut^(*),

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Several immunoregulatory abnormalities of EBV-induced B cell activation have been demonstrated in RA, suggesting that EBV may have a pathogenetic role in RA. Kahan et al. (Arthritis Rheum. 28, 961, 1985) demonstrated that RA patients (pts) could be divided into subgroups having a normal or a defective T cell regulation of IgM secretion by EBV-activated B cells. The activation of B cells by EBV is mediated through the EBVR, which was recently identified as gp 140, the C3d receptor (CR2) (Frade et al., Proc.Natl. Acad.Sci.USA, 82, 1490, 1985). In the present investigation, we studied the relationship between the defective T cell regulation of EBV-induced B cell activation and the expression of EBVR on LCL from 23 RA pts. 10 pts (group I) and 13 pts (group II) had a normal or defective suppressor T cell function (mean suppression of IgM secretion: 75% vs. 10% at 12 days of cultures, respectively). EBVR expression on LCL was assessed using a rabbit anti gp 140 IgG and 125-I protein A (RIA) and expressed as a percentage of maximal binding on Raji cells. Patients with defective T cell suppression of IgM secretion by EBV-activated B cells (group II) had a significantly higher EBVR expression on LCL (mean \pm SD, 50 \pm 23) than pts with a normal T cell suppression (mean \pm SD, 29 \pm 13) ($p < 0.05$). These data suggest a relationship between T cell suppression defect and increased EBVR expression on LCL from RA patients.

3.44.39

HLA-DR PHENOTYPES AND IMMUNE RESPONSE TO COLLAGEN IN RHEUMATOID ARTHRITIS. Carlos M.Vullo, Susana A.Pesoa, Carlos M. Onetti and Clelia M.Riera. Lab.Histocompatibilidad, Cátedra de Inmunología. Fac.C.Médicas y C.Químicas. Córdoba, Argentina.

Increasing evidence has been accumulated for the influence of genetic factors in the pathogenesis of rheumatoid arthritis (RA) in conjunction with the presence of immune reactivity to collagen. In the present study HLA-DR antigens were examined in 88 patients with classical RA and 52 normal individuals. Fifty eight per cent of patients with RA and 28 per cent of normal were DR4+ ($p < 0.01$). DR4 phenotype was significantly increased in patients with severe disease stage (III-IV) as defined by the criteria from the ARA in contrast to those showing a mild disease stage (I-II) ($p < 0.05$).

Furthermore, cells from the arthritic patients (n=55) produced leukocyte inhibitory factor in response to type I collagen ($p < 0.005$) and proteoglycans ($p < 0.025$) when compared with cells from normal individuals (n=30). Type II collagen did not induce activity consistently. Antibody against type I collagen were detected in 45% of 88 patients using ELISA method. The cellular immune response was not associated to any particular HLA-DR antigen, not either to the disease stage or severity.

The phenomena observed in this study indicate that DR4 antigen would predispose to a more severe disease and that immunity to type I collagen and proteoglycans is one of the features of RA showing that they are not in itself sufficient for the expression of the disease.

3.44.41

T CELL LINES DERIVED FROM RHEUMATOID SYNOVIUM.

J S H Gaston, J Solovera, S Strober, Div. of Immunology, Stanford Univ. Medical Center, U.S.A.

Synovial biopsies were obtained from 9 patients with seropositive rheumatoid arthritis; tissue fragments were cultured for 4-6 days in the presence of IL-2 and the resulting T lymphoblasts expanded using autologous Epstein-Barr virus-transformed cells (EB-LCL), RA synovial fluid (SF) and IL-2. These T cell lines, which contain cells of both helper and cytotoxic phenotype, have been maintained continuously for 8 months; sub-lines/clones were obtained by limiting dilution. All lines showed a proliferative response to autologous, but not allogeneic EB-LCL, which was inhibited by HLA-DR-specific monoclonal antibodies. In the presence of added IL-2 a response to non-EB virus transformed autologous stimulator cells was also seen. Specific cytotoxicity directed at autologous EB-LCL was also found with some T cell lines. These observations suggest that both EBV-specific and auto-reactive T cells may be derived from RA synovium.

T cell proliferation could be augmented by the addition of 1% RASF; this was due to a lymphokine-like activity of SF, rather than the recognition of an antigen in SF, since T cells which only proliferated in the presence of SF were not found.

3.44.38

MONOCLONAL ANTI-Ia ANTIBODIES SUPPRESS THE FLARE UP OF ANTIGEN INDUCED ARTHRITIS IN MICE. Maries F. van den Broek, Wim B. van den Berg, Levinus B.A. van de Putte, Dept. of Rheumatol. Univ. of Nijmegen Geert Grooteplein zuid 8, 6525 GA NIJMEGEN, the Netherlands.

Intravenous injection of mBSA in mice with an unilateral mBSA induced arthritis causes a flare up of the inflammation in the arthritic but not in the contralateral joint. To study the dependency on class II antigens, C57Bl/10 (H-2b) and CBA (H-2k) mice were treated with anti-Iab (HB26) and anti-Iak (2-2-1) antibodies on day -3, -2, -1, 0 or only at day 0 before antigen challenge. Four injections with HB26 completely suppressed the flare up in C57Bl/10 mice, the same results were seen with 2-2-1 in CBA mice. One injection partly suppressed the flare up in both strains, whereas four injections with the haplotype-nonspecific antibodies had no influence on the flare up. Injections with HB26 completely suppressed the DTH but not the RPA reaction in C57Bl/10 mice, indicating that anti-Ia antibodies have an effect on lymphocyte-dependent but not on antigen-antibody-dependent inflammatory phenomena. The flare up is dependent on Ia antigens suggesting a role for the interaction between APCs and T lymphocytes in this process.

3.44.40

INDUCED Ig AND RF SYNTHESIS BY SYNOVIAL FLUID B LYMPHOCYTES IN RHEUMATOID ARTHRITIS: ROLE OF SYNOVIAL FLUID T SUBSETS. J. Petersen & C. Heilmann, Lab. of Medical Immunology TA 7544, Rigshospitalet Univ. Hospital, Copenhagen, Denmark.

Plaque forming cell (PFC) assays were employed to quantify IgM, IgG, IgM-RF and IgG-RF production *in vitro* by blood and synovial fluid (SF) lymphocytes from 15 patients with rheumatoid arthritis (RA). Pokeweed mitogen (PWM)-stimulated blood lymphocytes yielded after 6 days median 15 IgM-RF-PFC and 12 IgG-RF-PFC/10⁶, accounting for about 0.5% of total IgM- and IgG-PFC. Due to excess of T8+ cells among SF lymphocytes, SF T cells were removed and replaced with autologous blood T cells to obtain significant PFC numbers among PWM-stimulated SF B cells. Under these conditions, PWM-stimulated SF B cells yielded median 40 IgM-RF-PFC and 440 IgG-RF-PFC/10⁶, accounting for about 8-9% of total IgM- and IgG-PFC. Next, purified T4+ and T8+ T cells from blood or SF were cultured with autologous blood B cells in the presence of PWM. In most patients, the ability of SF T4+ cells to increase Ig and RF production exceeded that of blood T4+ cells. The Ig production by blood lymphocytes co-cultured with autologous SF T8+ cells was suppressed to the same degree as seen with blood T8+ cells. About 1/3 of T4+ and 1/2 of T8+ SF T cells were Ia+ as opposed to about 5% of blood T subsets. Thus, Ia+ helper and suppressor/cytotoxic cells may modulate the activation of synovial B cells in RA.

3.44.42

ELEVATED LEVELS OF TACHYKININ-LIKE IMMUNOREACTIVITY IN JOINT FLUIDS FROM PATIENTS WITH RHEUMATOID ARTHRITIS.

P.Devillier*, B.Weill*, C.Menkès*, M.Renoux*, P.Pradellest. (*)Lab.Immunologie, (**)Sec de Rhumatologie, C.H.U. Cochin Port-Royal, Paris; (+)L.E.R.I., Section S.P.I., Commissariat à l'Énergie Atomique, Saclay, France.

Substance P (SP), a tachykinin released from the peripheral terminals of primary unmyelinated afferent fibers, has been involved in rat experimental arthritis. In the past two years, two other mammalian tachykinins have been identified: substance K (SK) and neuromedin K (NK). On the other hand, it has been shown that distal joints, which are the most frequently and severely damaged in rheumatoid arthritis, are also the most innervated joint.

Assuming that tachykinins might be involved in the inflammatory process of rheumatoid arthritis, tachykinin-like immunoreactivity was assayed in the synovial fluids from 12 patients with rheumatoid arthritis and 3 patients with osteoarthritis. An enzymeimmunoassay was developed using an antiserum raised against the C-terminal pentapeptide of SP and cross-reacting to varying extents with the similar C-terminal amino acid sequences of SK and NK. This assay showed a higher rate of tachykininlike reactivity in inflammatory fluids (3.98 \pm 1.64 ng/ml*) than in non-inflammatory fluids (1.91 \pm 0.56 ng/ml*) ($p < 0.001$, student's t test). Structure-activity relationship studies clearly indicate that the biological activity of tachykinins is born by the C-terminal end. Since the antisera detected the C-terminal fragments of the various tachykinins, the reactivity tested in this assay reflected the concentrations of biologically active tachykinins. This report suggests a possible role of neuropeptides in the development of some joint inflammatory processes as in rheumatoid arthritis. (*:mean \pm 1 SD, values are expressed in SP equivalents).