visual field mean deviation <−2 dB was arbitrary and differed significantly from our understanding of the neuropathological basis of visual field damage specific to NTG.

Araie and coworkers have indicated that NTG and the ordinary primary open angle glaucoma or high tension glaucoma (HTG) showed significantly different visual field damage.[3] Visual field defects in NTG are more localised and predominant in the lower hemifield, whereas HTG has significantly more diffuse visual field damage.[4–6] It has been demonstrated that mean deviation in perimetry is good measure for assessing the more diffuse visual field damage characteristic of HTG but not as good for pinpointing a localised defect such as that seen in NTG.[7 8] Instead, pattern standard deviation or corrected pattern standard deviation were suggested as alternative indicators in representing the focal visual field defect in NTG.[7 8] As a result, the authors' conclusion about the relationship between NTG and systemic sclerosis may be based on an erroneous visual field index (mean deviation), which is neither sensitive nor specific for NTG.

Moreover, it should be pointed out that Allanore *et al* have adopted another arbitrary means of defining the IOP of the subjects recruited, which again showed marked disparity from our usual practice. The authors did not explain why phasing of the IOP was not undertaken given the fact that IOP shows diurnal variation, especially prominent in glaucomatous subjects such as those with NTG.[9] Recording of only one IOP measurement may not be sufficient owing to the influence of this confounding factor.

Appropriate case definition lies at the heart of every epidemiological research on glaucoma and any deviation from the consensual definitions may inevitably skew or even imperil the validity of the data.[9] In the interest of readers, we would be most grateful if the authors can provide us with more information about the rationale for the methodology used.

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Authors' reply

We thank Drs Chan and Liu for their comments about our article evaluating ocular glaucomatous changes in systemic sclerosis (SSc).

High intraocular pressure (>21 mm Hg) is undoubtedly known to be the main risk factor associated with glaucoma[1]; however, substantial evidence was provided recently to support a key role of vascular abnormalities in the pathogenesis of glaucoma. In particular, patients with normal tension glaucoma, who do not have the main risk factor of developing glaucoma (increased intraocular pressure), may also develop optic neuropathy, and numerous recent studies support the hypothesis that these lesions are associated with vasculopathies.[2–4] These findings led us to investigate the prevalence of glaucomatous changes in SSc, a disease which is strikingly associated with generalised vascular involvement.

Although primary open angle glaucoma is well defined, normal tension glaucoma is more difficult to diagnose. Independently of intraocular pressure, glaucomatous changes are supported by optic disc cupping together with visual field defects.[1] Thus, for the purpose of our comparisons between groups, we had to define cut off values for these two variables. For optic disc cupping, we chose a cut off point based on reported data[5]; we defined mild abnormalities as a c/d >0.3 and severe involvement as a c/d >0.7. For visual field, we also chose a mean difference <−2 dB according to reported data. Thus, the significant differences between SSc and matched controls for these measures allow us to suggest that patients with SSc have glaucomatous abnormalities as compared with our controls. Although there is no consensual definition of NTG, these results clearly suggest that patients with SSc have glaucomatous propensity. The continuing prospective standardised follow up of our patients and other series will quantify the precise risk factor of SSc for normal tension glaucoma.

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CD68 is not a macrophage-specific antigen

The article of Kunisch *et al* discussing a cross reactivity of allegedly macrophage-specific anti-CD68 antibodies with fibroblasts and activated endothelial cells demonstrates amply that these antibodies should not be used for the identification of macrophages.[1] Yet they have been used for this purpose in nearly all medical disciplines, particularly in vascular diseases. In 1990 we observed that some neointimal cells in experimental transplantation atherosclerosis, human native atherosclerosis, and experimental native atherosclerosis had reacted with both presumptive macrophage-specific antibodies (RAM11, HAM56) and an antibody against muscle actin (HHF35).[2] In 1997, Andreeva *et al* demonstrated that the very same human intimal and neointimal cells were immunopositive, both with anti-macrophage (CD68, HAM56) and anti-muscle actin (asm-1, HHF35) antibodies.[3] On the basis of these findings, these authors formed a hypothesis that the macrophage markers involved in these reactions were not indicative of cell histogenesis but of phagocytosis. Neither our observation[2] nor the demonstration of Andreeva *et al*[3] had any influence on the practice of macrophage identification by the above mentioned antibodies.

Today, I share Kuhn's opinion[4] that the acceptance or rejection of new scientific ideas depends on their relationship to existing paradigms. If they are in agreement with them they are accepted, but if they contradict them they are usually ignored. When the immunohistochemical identification of macrophages was originally proposed there was no existing paradigm in this field and its authors presented their methods against no substantial opposition. My observation that an unreasonably high amount of macrophages had been identified with new monoclonal antibodies in comparison with previously used electron microscopy was disregarded.[5] Rare articles describing the reactivity of the above mentioned anti-macrophage antibodies with other cell phenotypes in other medical disciplines were also neglected.

Kuhn described the scientific process as a conflict, in which less satisfactory paradigms are replaced successively by better ones.[4] There is only one way which guarantees the correctness of individual paradigms: a strict observance of the facts. For example, an immunological injury induces an intimal thickening composed only of ''macrophages''

identified by "macrophage-specific" antibodies in a hypercholesterolaemic rabbit. Serial sections show, however, that the cells in question are smooth muscle cells manifesting both macrophage and muscle actin antigens.[2 6] Because macrophages cannot produce muscle actin, the cells must be smooth muscle cells phagocytising lipids, and the paradigm of macrophage-specific antigens should be replaced by the paradigm of phagocytotic antigens.

In the article by Kunisch *et al*,[1] it would be interesting to know whether the extent of the overlap between "macrophage" and fibroblast markers in individual patients correlates with some measures of their rheumatoid arthritis, such as synovium hypertrophy, pannus formation, cartilage erosion, and bone destruction. Also, is there a relationship between anti-CD68 positive synovial fibroblasts and contingent dyslipidaemias in rheumatoid arthritis? In vascular diseases, "macrophage-specific" antibodies react with smooth muscle cell phagocytising lipids.[3] A similar process in which phagocytising synovial fibroblasts would become immunopositive with anti-CD68 antibodies may take place in rheumatoid arthritis.

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Authors' reply

We sincerely thank Dr JT Beranek for his thoughtful letter and his comments on our report. He supports our view that CD68 is not a specific marker for macrophages but rather an antigen indicative of phagocytosis,[1] as also expressed in several studies in atherosclerosis and other areas.[2–8] Our own continuing experiments also support an interrelationship between phagocytosis and the expression of CD68 proteins. After phagocytosis of conventional phosphatidylcholine/phosphatidylglycerol/cholesterol liposomes (24 hours), the human monocytic cell line THP-1 increased the expression of the CD68 epitope recognised by the monoclonal antibody (mAb) EBM11, but not the CD68 epitope recognised by the mAb KP1 (fig 1). In contrast, only marginal effects were seen in human synovial fibroblasts at this time.

To determine whether this finding is based on redistribution,[7] conformational change, or altered glycosylation pattern[8] of the CD68 molecule, or on (trans)differentiation of the

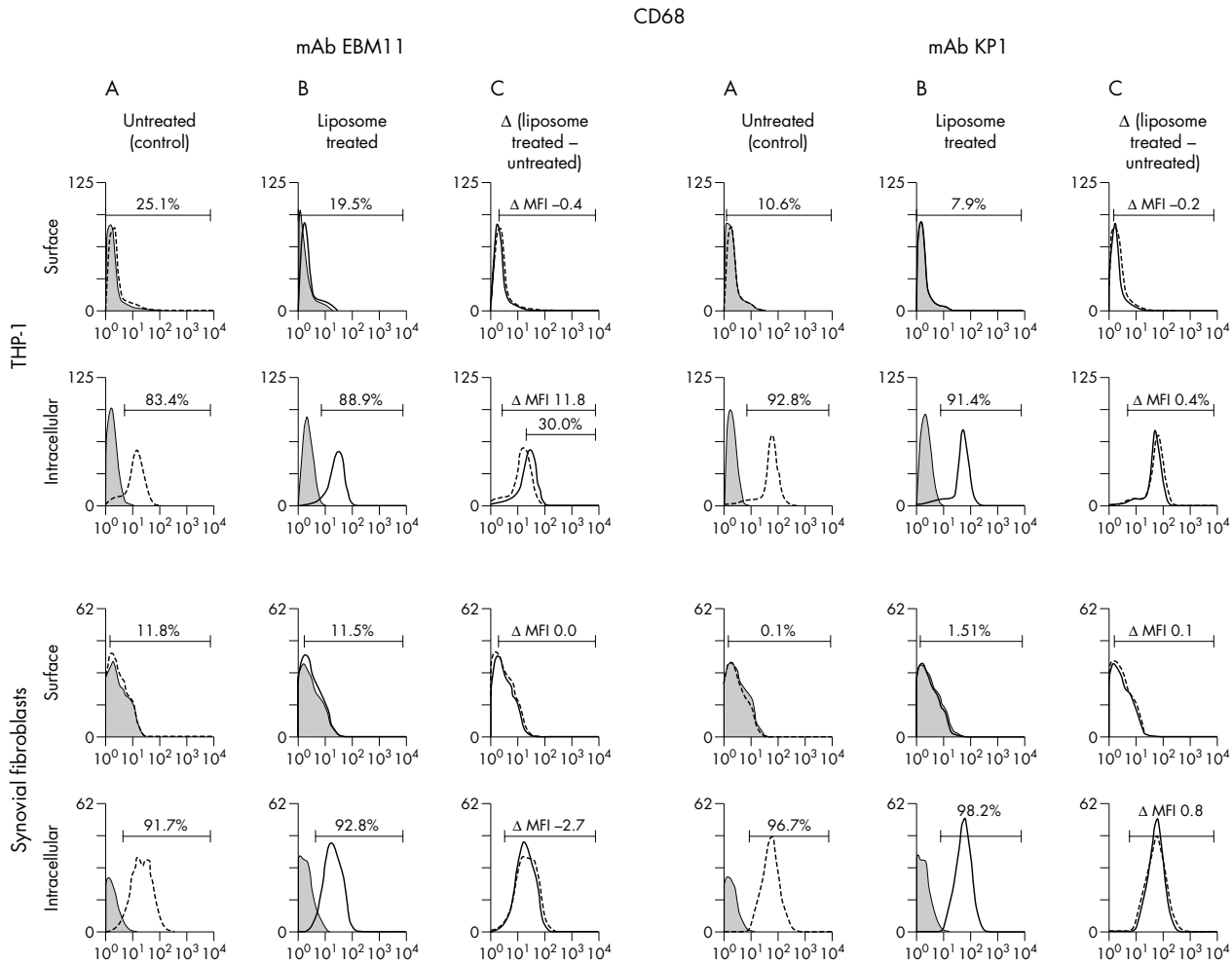

Figure 1 CD68 expression (mAb EBM11 or KP1, surface and intracellular) of THP-1 cells and synovial fibroblasts after incubation with phosphatidylcholine liposomes for 24 hours (dipalmitoylphosphatidylcholine, dipalmitoylphosphatidylglycerol, cholesterol at a molar ratio of 50:10:40; mean size 495 nm). THP-1 cells were incubated with liposomes in suspension for 24 hours. Synovial fibroblasts were allowed to attach for 24 hours followed by incubation with liposomes for 24 hours. Thereafter, CD68 expression (mAb EBM11 and KP1, surface and intracellular) was determined by flow cytometry (A and B) isotype control – solid line; specific antibody – dashed/solid line; (C) CD68 expression in untreated cells – dashed line; CD68 expression in liposome treated cells – solid line; *x* axis: fluorescence intensity; *y* axis: counts).

cells,[6] requires further study. The observation that different types of non-macrophage-like cells express the ''macrophage'' marker CD68 in several diseases, clearly has the consequence that these ''macrophage-like'' cells have to be more thoroughly identified using other cell-type specific markers and the appropriate technique and fixation. We also agree with Dr Beranek's point that the revival of morphological or ultrastructural techniques in connection with modern immunohistology/in situ hybridisation is essential in clarifying some of these controversial findings.

As regards the correlations between the extent of overlap between ''macrophages'' and fibroblast markers in individual patients and their measures of clinical disease or the contingent dyslipidaemias in rheumatoid arthritis, we can only provide a partial answer. When patients with rheumatoid arthritis and osteoarthritis were analysed together by the Spearman rank correlation, the percentage of synovial fibroblasts positive by FACS staining for the anti-CD68 mAbs KP1 or EBM11 showed a significant negative correlation with disease markers such as the number of fulfilled American Rheumatism Association criteria[9] or the number of leucocytes in peripheral blood (maximum $r_s = -0.715$; $p = 0.006$; $n = 13$). Also, the percentages of synovial fibroblasts positive for the KP1 or EBM11 epitopes of CD68 showed a highly significant positive correlation with each other ($r_s = 0.951$; $p = 0.000$; $n = 13$), as well as with other fibroblast markers like Thy-1 (CD90) or prolyl-4-hydroxylase (maximum $r_s = 0.750$; $p = 0.002$; $n = 14$).

Finally, we thoroughly agree with Dr Beranek's thoughtful considerations on the interrelationship between ignoring new scientific evidence and the persistence of incorrect paradigms. His recommendation to return to the strict observance of facts, indeed an incontrovertible basis for scientific conduct, should encourage a discussion on the influence of the human factor[10] in scientific peer review.

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FORTHCOMING EVENTS

VIth European Lupus Meeting

3–5 March 2005; Royal College of Physicians, London, UK
Contact: Julia Kermode, Conference organiser of the British Society of Rheumatology
Email: Julia@Rheumatology.org.uk

Thirteenth Intensive Applied Epidemiology Course for Rheumatologists

7–11 March, 2005; Manchester, UK
No previous experience in epidemiology is required. Residential course limited to 20 places
Contact: Ms Lisa McClair, ARC Epidemiology Unit, University of Manchester, Oxford Road, Manchester M13 9PT, UK
Tel: +44 (0) 161 275 5993
Fax: +44 (0) 161 275 5043
Email: Lisa.mcclair@man.ac.uk

International Society for the Study of the Lumbar Spine Instructional Course

27, 28 March 2005; Nairobi, Kenya
Controversies in diagnosis and treatment of lumbar spine conditions
Contact: Shirley Fitzgerald, 2075 Bayview Avenue, Room MG323, Toronto, Ontario, Canada M4N 3M5
Tel: 416 480 4833
Fax: 416 480 6055
Email: shirley.fitzgerald@sw.ca

BSR Annual Meeting 2005

19–22 April 2005; ICC, Birmingham, UK
Joint meeting with the German Society for Rheumatology
Contact: BSR, 41 Eagle Street, London WC1R 4TL, UK
Tel: +44 (0) 20 7242 3313
Fax: +44 (0) 20 7242 3277

EULAR 2005

8–11 June 2005, Vienna, Austria
Contact: EULAR Secretariat
Tel: +41 1 383 96 90
Fax: +41 1 383 98 10
Email: secretariat@eular.org
Website: http://www.eular.org/eular2005

Future EULAR congresses

21–24 June 2006; EULAR 2006; Amsterdam, The Netherlands
13–16 June 2007; EULAR 2007; Barcelona, Spain
11–14 June 2008; EULAR 2008; Paris, France