TABLE 1 Binding of immune complexes

Immune complexes	Binding to apoptotic neutrophils mean fluorescence	Reduction in binding %
None	3.4	
Uncentrifuged	92.3	
14000 × *g* for 20 min	86.8	2.4
300000 × *g* for 60 min	16.0	82.0

human lung mast cells, requires demonstration that there were no IgE aggregates in the IgE preparations. The authors proposed that centrifugation at 14,000 × *g* for 20 min would remove any aggregated IgE, but it is unlikely that this was effective. We have examined the effect of centrifugation on binding of IgG-containing immune complexes to the low-affinity IgG receptor FcγRIIA on apoptotic neutrophils. Fluorescent immune complexes were generated by combining monoclonal mouse IgG1 anti-fetuin with fluorescein-conjugated fetuin in conditions of antigen excess, and then subjected to centrifugation at 14,000 × *g* or 300,000 × *g* for 20 or 60 min, respectively. Binding of immune complexes in the post-centrifugation supernatants to apoptotic human neutrophils was measured by flow cytometry as previously described [4]. The results are presented in table 1.

These results demonstrate that centrifugation at 14,000 × *g* for 20 min does not effectively deplete immune complexes. Centrifugation at 300,000 × *g* for 60 min removes the majority of immune complex binding, but this would be inadequate for studies of cellular activation in which even immunoglobulin dimers may induce functional responses [5]. In contrast to centrifugation, size-exclusion chromatography would be a reliable method for purifiying monomeric immunoglobulin E for use in mast cell activation studies.

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From the authors:

We are writing in response to the letter to the *European Respiratory Journal* by S. Hart and I. Dransfield regarding our recent paper [1]. We thank them for bringing to our attention that the centrifugation of our immunoglobulin (Ig)-E preparation at 14,000 × *g* for 20 min might be insufficient to remove IgE aggregates or multimers. We are, however, confident that the human myeloma IgE that we used in our experiments is free from such complexes (a belief that is shared by the manufacturer Calbiochem Novabiochem, Nottingham, UK).

Purified Ig preparations often contain immune aggregates. This has been demonstrated in several rodent studies [2–4]. These studies have also demonstrated that the efficacy of such IgE aggregates at initiating a response in mast cells is very poor compared with that of monomeric IgE.

These studies used high-performance liquid chromatography [2, 3], or size-exclusion chromatography [4] to ensure that their IgE preparations were truly monomeric, and recorded an array of responses with the monomeric forms. There are many recent studies which agree that monomeric IgE induces an array of responses in mast cells [5–8]. In our study we used IgE that was purified from the plasma of a myeloma patient. These preparations, therefore, are paraproteins that, as evidenced in IgE multiple myeloma, do not readily form dimers such as IgA or pentamers such as IgM, in the absence of a soluble antigen. The preparations have been assayed by the manufacturer using immunoelectrophoresis and produced a single arc, which further suggests a lack of aggregates or multimers. Any affinity for binding epitopes on the IgE molecules themselves would be very low. However, if there are intermolecular interactions with the IgE molecules at the receptor sites on the mast cell surface, as has been suggested in a recent mathematical model [9], this would not disprove the theory that monomeric IgE is an important activator of mast cell secretion, as the cross-linking of IgE by such interactions would occur *in vivo* at the same concentrations (the concentrations we used were experienced *in vivo*).

Therefore, we are not mimicking allergen exposure, but instead representing the physiological response to increased serum immunoglobulin E.

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Long-term oxygen therapy

To the Editors:

The topic of long term oxygen therapy (LTOT) has been a great interest to our group, beginning with our first report in 1967 [1]. Here, we showed a marked increase in exercise capability with use of ambulatory oxygen (AO) in selected patients with advanced chronic obstructive pulmonary disease (COPD). By contrast, the carefully carried out, randomised, controlled study of AO in oxygen-dependent patients, recently published in the *European Respiratory Journal*, concludes that AO is not associated with increased walk tolerance or improved quality of life [2]. The landmark Nocturnal Oxygen Therapy Trial (NOTT), showed improved quality of life with AO and a statistically significant improvement in survival, compared with stationary oxygen (SO). AO patients received oxygen for a mean of 17.7 h compared with 11.8 h for SO patients. Thus, the differences could either be due to the duration of oxygen therapy or the method [3]. Furthermore, a re-analysis of the NOTT revealed that those patients who could improve their exercise level prior to randomisation and received AO, had a highly significant improvement in survival, compared with SO patients with low levels of exercise capability on training. AO patients with good exercise capacity had far fewer hospitalisations, compared with SO patients with less ability to exercise [4].

In the study by LACASSE *et al*. [2], patients who were already receiving oxygen for $\geqslant$3 months were selected. Herein lies my criticism. After some four decades of LTOT studies (plus 2 yrs of personal use), I must comment that patients given SO rapidly adjust to the limitations imposed by their system. They tend to avoid going outside the home, even when a portable system is given to them. It has been documented that patients with COPD who receive LTOT have a much lower level of domiciliary activity, compared with COPD patients of equal severity who do not receive LTOT [4].

The patients in the study by LACASSE *et al*. [2] did not go outside the home for >2 h per day, and most often did this without their portable cylinder. In my experience, this is due to the fixation on use of the oxygen from the home-base stationary system. Furthermore, the patients did not use their ambulatory system in conjunction with prescribed exercise. I believe the previous information explains the lack of improvement in both groups in this study. Thus, this study is not designed to prove that AO is not beneficial to activity, as the authors conclude.

In conclusion, new studies are needed in oxygen-naïve patients for long-term oxygen therapy in chronic obstructive pulmonary disease, given in conjunction with exercise as a component of a pulmonary rehabilitation programme, as was originally suggested [6].

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