Clin Exp Allergy 2005; **35**:117–121 doi:10.1111/j.1365-2222.2005.02173.x

# EDITORIAL

# Airway remodelling in asthma: models and supermodels?

H. H. Kariyawasam and D. S. Robinson

Although currently anti-inflammatory treatments are effective for most of the 5 million patients treated in the UK they do not completely control symptoms, and a minority of asthmatics continue to experience severe debilitating disease [1]. Of note, although inhaled corticosteroids reduce the Th2-type eosinophilic airway inflammation and airway hyperresponsiveness (AHR) that characterize asthma, they do not restore either variable to normal [2]. For this reason attention has been focused on structural changes in the asthmatic airway termed airway remodelling. These changes include epithelial damage and goblet cell hyperplasia [3], increased airway smooth muscle [4] and recruitment and activation of myofibroblasts [5]. Increased deposition of collagens and other extracellular matrix proteins such as tenascin and fibronectin occurs both in the reticular basement membrane (RBM) [6] and throughout the bronchial mucosa and this structural remodelling is accompanied by new blood vessel formation [7]. The clinical consequences of airway remodelling in asthma remain uncertain but contributions to AHR and fixed airflow obstruction have been suggested. Mathematical modelling predicts that airways with increased smooth muscle narrow to a much greater extent than airways with less smooth muscle volume for a given degree of circumferential smooth muscle shortening [8]. Asthmatic subjects show accelerated decline in lung function over time compared with non-asthmatic subjects, and this loss of lung function is more marked in asthmatic smokers [9, 10]. Although duration and severity of asthma are the most important risk factors for development of fixed obstruction the highest rate of loss of lung function is seen during the early course of the illness. Interestingly, remodelling changes are seen early in childhood and may even pre-date the onset of symptoms [11]. The impact of current anti-inflammatory treatment on remodelling is controversial. Although partial reversal of remodelling has been reported with inhaled corticosteroid treatment, responses are seen after prolonged therapy [12] and are not confirmed in all studies. The initial rapid improvement in AHR in asthma with inhaled corticosteroid treatment may be explained by reduction in airway inflammation [13], but additional improvements over many months may reflect changes in remodelling. Important questions about airway remodelling in asthma remain unanswered (see Fig. 1). These include:

(1) What is the link between airway inflammation and airway remodelling? To what extent does remodelling represent a repair response to inflammation and to what extent an intrinsic abnormal response to insults such as viral infection or allergen inhalation?
(2) What are the clinical consequences of airway remodelling: is it an important therapeutic target?
(3) Which mediators are involved in the various changes of remodelling and how can treatments be devised to prevent or reverse structural changes?

Only by examining the effect of specific interventions which impact on remodelling will the real clinical and physiological consequences of this process become clear.

There are no non-invasive methods of assessing airway remodelling at present and human airway research is limited thus mainly to morphological and *in-vitro* experimental work constrained further by ethical implications. Nonetheless recent bronchoscopic studies have provided important insights into the link between eosinophilic inflammation and extracellular matrix (ECM) deposition in asthma [14]. Eosinophils have been associated with fibrosis in a number of settings [15] and recent *in vitro* data suggest that production of TGF-β by eosinophils can cause differentiation of fibroblasts to myofibroblasts and initiate synthesis of ECM proteins such as tenascin-C [16]. In addition, anti-IL-5 treatment in moderate asthmatics reduced airway eosinophil infiltration by 50%, and also reduced expression of TGF-β by these cells. These changes were accompanied by significant reduction in sub-basement membrane deposition of the ECM proteins tenscin, lumican and procollagen III [17]. Further evidence linking eosinophilic airway inflammation with at least some aspects of airway remodelling came from the observation of increased RBM tenascin deposition 24 h after allergen challenge in asthmatics, together with nuclear localized of activated Smad2 suggestive of active TGF-β signalling in fibroblasts and epithelial cells as an acute response to allergen exposure [18].

Animal models of allergic airway inflammation have been useful for defining potential roles for specific cytokines and other mediators by use of knockouts, transgenics and *in vivo* administration of antibodies or pharmacological inhibitors [19, 20]. It is important to interpret data from such models with a degree of caution since findings in acute mouse models may not reflect what happens in different mouse strains using different challenge protocols never mind the chronic inflammatory changes in human asthma. Nonetheless, recent development of chronic inhaled challenge models in mice by a number of groups has allowed the recapitulation of many of the features of airway remodelling seen in human asthma, and these tools may thus prove useful in identifying research questions for study of human disease.

Several groups have reported on studies of airway remodelling in mouse models, which have been used to study the relationship between specific cytokines and cell types with aspect of the remodelling process. There are some differences

 

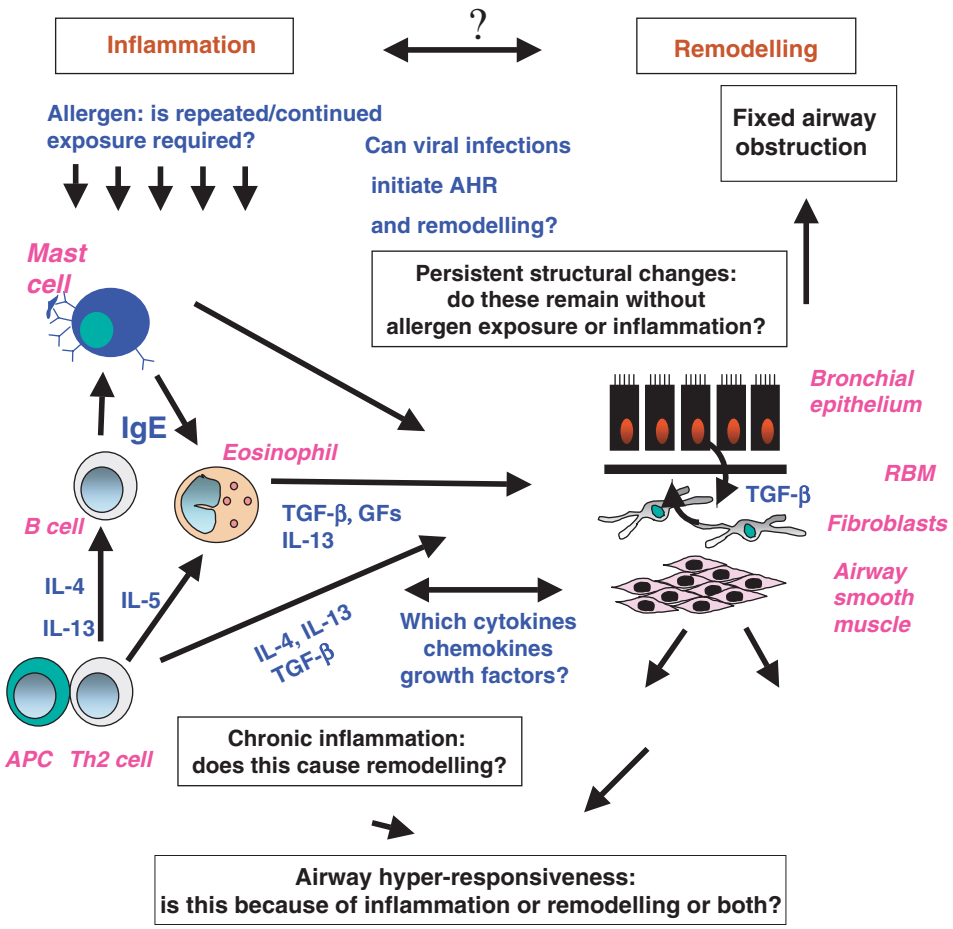


**Fig. 1.** Airway inflammation and remodelling in asthma: important questions. Cells and mediators implicated in airway inflammation and remodelling are shown: in particular current evidence from human studies and animal models implicate eosinophils and TGF-β in remodelling changes. Which mediators and growth factors are important, how inflammation and structural changes are linked and what controls this link remain important questions for both animal models and human studies. In addition the functional and clinical consequences of remodelling remain to be determined.

in findings from these studies, which may reflect different protocols used. Leigh et al. [21] studied BALB/c mice sensitized with intraperitoneal ovalbumin (OVA) in aluminium hydroxide (a Th2-promoting adjuvant used in most mouse models of allergic airway inflammation). In the chronic challenge model mice then received intranasal OVA for 2 days every 12 days over six occasions, and changes in airway inflammation, AHR and remodelling were examined at 24 h, 2, 4 and 8 weeks after the last challenge. AHR was measured as change in airway resistance (measured by tracheal cannulation) to intravenous methacholine. These investigators found persistence of AHR, smooth muscle hypertrophy, mucus hypersecretion and airway fibrosis up to 8 weeks following the last challenge, whereas airway eosinophilia and IL-13 expression were increased at 24 h after challenge then returned towards baseline values. This model has subsequently been used to show that sustained AHR to chronic challenge was not seen in IL-4 or IL-13 knockout mice but did develop in the absence of IL-5 [22]. If blocking antibodies were given after the last challenge, blocking IL-13 [23] or depleting CD4 or CD8 T cells [24] did not affect persistence of established remodelling or AHR. McMillan and Lloyd [25] studied BALB/c mice sensitized by two intraperitoneal injections of OVA (10 μg) in alum, then six daily OVA inhalations on days 18–24, followed by aerosol OVA challenge for 3 days of each week for 2 weeks, 4 weeks or 6 weeks. Airway changes and AHR (by measurement of Pen H) were assessed 24 h after each set of challenges or at 80 days (4 weeks after the last challenge). In this model peribronchial fibrosis, airway smooth muscle (ASM) deposition and IL-4 expression persisted to day 80, but AHR fell back to baseline as did airway eosinophilia, IL-5 and IL-13. Increased TGF-β protein expression was seen at days 35 and 55 (mainly in macrophages, not eosinophils) but did not persist to 80 days. A recent report from Johnson et al. [26] uses a different model. These investigators gave BALB/c mice intranasal house dust mite extract (25 μg protein) for 5 days a week for up to seven consecutive weeks. This protocol, without prior sensitization, resulted in marked Th2 and eosinophilic airway inflammation, and total cell and eosinophil counts returned to baseline within 2 weeks of the last challenge. There was also increased lung collagen deposition, which persisted up to 9 weeks after challenge, and smooth muscle staining which remained elevated at 9 weeks although falling slightly from post-challenge values. In this model AHR remained elevated 9 weeks after the last challenge but was less than that seen at 24 h after seven weeks of repeated challenge. Interestingly intranasal OVA without prior sensitization did not lead to allergic airway inflammation or changes or remodelling. Other reports have not detailed persistence of remodelling after chronic challenge, although the report by Cho et al. [27] states that ASM hypertrophy persisted for 1–3 months in their model. These authors used C57/Bl mice sensitized with 50 μg OVA in alum on days 0 and

12 then given intranasal OVA twice per week for 3 months. At 24 h after the last challenge they found increased airway eosinophils and lymphocytes, ASM, collagen deposition and expression of TGF-β (in this model localized to eosinophils and macrophages). IL-5 knockout showed attenuated airway collagen deposition and ASM (though not to baseline) together with reduced eosinophilia and TGF-β expression compared with wild-type control mice suggesting a role for eosinophils in remodelling. The model of McMillan and Lloyd [28] has recently been used to study the role of eosinophils by using GATA1 deficient mice which lack eosinophils: these mice showed AHR to acute airway challenge but failed to develop airway fibrosis or ASM hypertrophy in the chronic challenge model. In agreement with these findings an earlier report by Blyth et al. [29] had shown reduced RBM reticulin staining in BALB/c mice treated with anti-IL-5 antibody before each of three intranasal OVA challenges 40 days after a sensitization protocol with seven daily intraperitoneal injections of OVA without adjuvant. Other mouse models have been used to study airway remodelling, such as instillation of aspergillus spores into the airway of previously sensitized mice (which led to persistent airway fibrosis) [30], and most notably by selective transgenic expression of cytokines including IL-4, 5, 9, 11 and 13 under promoters selective for airway epithelial expression [31–35]. However, although these identify potential actions of certain cytokines they are a long way from human asthma. To date there are no reports examining effects of depletion of mast cells, induction of epithelial changes (such as over expression of ADAM33) or smooth muscle hypertrophy but the models should allow further dissection of potential players in remodelling. The effects of protocol and mouse strain were studied by Shinigawa and Kojima [36]. These investigators studied intraperitoneal sensitization with OVA and alum followed by chronic inhaled (× 5 per week for 1–4 weeks) or intranasal OVA challenge (× 3 per week at three four weekly intervals) protocols in four mouse strains: A/J, BALB/c, C57/BL6 and C3H/HeJ. Changes were studied at 24 h after the last challenge so it is difficult to comment on persistence of remodelling and AHR. However they report that the A/J strain showed long-term eosinophilic inflammation, AHR airway wall thickening and fibrosis with a chronic intranasal challenge with lesser changes seen in BALB/c and no persistence of AHR or inflammation with chronic challenge in C57/Bl6 or C3H/HeJ mice. Inhaled OVA challenge lead to AHR and airway eosinophilia in all strains but this persisted with challenges 5 days a week for 4 weeks only in the A/J mice while inflammation and AHR diminished over time in other strains. While differences in findings about persistence of AHR and remodelling after resolution of airway inflammation may reflect differences in mouse strain, protocol and measurement of AHR they are frustrating if one is trying to gain insights of human disease. Clearly strain differences imply variable genetic sensitivity to persistence of remodelling and this may also true for humans.

In this issue of the journal Snibson et al. [37] describe a model of chronic airway remodelling in sheep in response to house dust mite (HDM) inhalation over a 6-month period. The group has previously established a sheep model using lung segmental allergen challenge with HDM to mimic human inflammatory responses and the current model is an extension of this study. The advantage of using HDM is that it has obvious relevance to human asthma and is not an allergen that would be encountered by sheep outside a protocol allergen exposure setting. This is in contrast to other inflammatory models set up in sheep using allergen that the animal is already sensitized to such that changes detected in such a model cannot be interpreted in relation to a specific exposure history. Using sheep that displayed high serum HDM specific IgE levels and a segmental lung allergen challenge protocol using fibre-optic bronchoscopy (FOB) the authors performed allergen challenge twice weekly for 3 months and then weekly for the final 3 months. A separate segmental lung lobe in the same sheep was used as an internal control in addition to three separate sheep that received saline-in adjuvant immunisation and saline only segmental challenge. Bronchoalveolar lavage via FOB was performed as part of the time course study and approximately a week after the final challenge killed for whole lung removal and histological analysis. The authors found that prolonged allergen challenge to sensitized allergen induces goblet cell hyperplasia, collagen deposition and smooth muscle hyperplasia but only in three of the seven sheep studied. The demonstration of eosinophil recruitment to the airway at 48 h after allergen challenge and the tissue localisation near epithelium and smooth muscle, both integral components of the epithelial–mesenchymal unit that is considered to drive the remodelling changes in humans is relevant. Eosinophil degranulation is seen with human asthma and is correlated to severity but such changes were not convincingly demonstrated here. Eosinophils were absent 8–9 days after the last allergen challenge and this suggests that it is persistent allergen exposure that maintains inflammation and may drive remodelling. It would be important to address this point by using the model to evaluate the reversibility and time course of such remodelling events in relation to inflammatory changes. Mast cell density was increased after challenge in the autopsy specimen. The authors do not comment on epithelial integrity or RBM thickening that is characteristic of human asthma.

What further insight can we obtain from such a model of airway remodelling? The authors point out that sheep lung innervation and blood supply is nearer that of humans than mice. However the current findings do not add significantly to information from murine models described above or from human studies. Reagents for intervention in sheep are limited compared with those available for mice. Such a model might be used for drug testing but it remains to be seen if it will significantly add to information from mouse and human studies. A further large animal study was recently published by Tran et al. [38] who studied Rhesus monkeys. The effects of sensitization (by subcutaneous injection of HDM (*Dermatophagoides farinae*) extract in alum with intramuscular pertussis toxoid followed by inhalation of house dust mite over six months after birth on airway smooth muscle were studied. Monkeys sensitized then exposed to HDM developed ASM hypertrophy, but these changes were not related to AHR or inflammation. One might argue that monkey models are closer to humans than sheep, but since one can also induce acute remodelling changes in humans by allergen challenge and sample the airways at bronchoscopy

[39], the utility of large animal models over human studies remains questionable.

Asthma is a chronic complex heterogenous condition and no animal model can ever mimic the human disease completely. Animal models provide valuable insights into the potential roles of complex molecular pathways in relation to disease phenotype, but ultimately studies of human asthma will be required.

## Acknowledgements

The authors thank Prof Barry Kay and Drs Clare Lloyd and Sarah McMillan for helpful comments on the manuscript. DSR is supported by a Wellcome Trust Research Leave Award to Clinical Academics.

## References

1 Barnes PJ, Woolcock AJ. Difficult asthma. Eur Respir J 1998; 12:1209–18.
2 Lim S, Jatakanon A, John M et al. Effect of inhaled budesonide on lung function and airway inflammation. Assessment by various inflammatory markers in mild asthma. Am J Respir Crit Care Med 1999; 159:22–30.
3 Ordonez CL, Khashayar R, Wong HH et al. Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. Am J Respir Crit Care Med 2001; 163:517–23.
4 Johnson PR, Roth M, Tamm M et al. Airway smooth muscle cell proliferation is increased in asthma. Am J Respir Crit Care Med 2001; 164:474–7.
5 Brewster CE, Howarth PH, Djukanovic R, Wilson J, Holgate ST, Roche WR. Myofibroblasts and subepithelial fibrosis in bronchial asthma. Am J Respir Cell Mol Biol 1990; 3:507–11.
6 Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. Lancet 1989; 1:520–4.
7 Li X, Wilson JW. Increased vascularity of the bronchial mucosa in mild asthma. Am J Respir Crit Care Med 1997; 156:229–33.
8 Moreno RH, Hogg JC, Pare PD. Mechanics of airway narrowing. Am Rev Respir Dis 1986; 133:1171–80.
9 Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. N Engl J Med 1998; 339:1194–200.
10 Ulrik CS, Backer V. Nonreversible airflow obstruction in life-long nonsmokers with moderate to severe asthma. Eur Respir J 1999; 14:892–6.
11 Baldwin L, Roche WR. Does remodelling of the airway wall precede asthma? Paediatr Respir Rev 2002; 3:315–20.
12 Lundgren R, Soderberg M, Horstedt P, Stenling R. Morphological studies of bronchial mucosal biopsies from asthmatics before and after ten years of treatment with inhaled steroids. Eur Respir J 1988; 1:883–9.
13 Haahtela T, Jarvinen M, Kava T et al. Effects of reducing or discontinuing inhaled budesonide in patients with mild asthma. N Engl J Med 1994; 331:700–5.
14 Kay AB, Phipps S, Robinson DS. A role for eosinophils in airway remodelling in asthma. Trends Immunol 2004; 25:477–82.
15 Xu X, Rivkind A, Pikarsky A, Pappo O, Bischoff SC, Levi-Schaffer F. Mast cells and eosinophils have a potential profibrogenic role in Crohn disease. Scand J Gastroenterol 2004; 39:440–7.
16 Phipps S, Ying S, Wangoo A, Ong YE, Levi-Schaffer F, Kay AB. The relationship between allergen-induced tissue eosinophilia and markers of repair and remodeling in human atopic skin. J Immunol 2002; 169:4604–12.
17 Flood-Page P, Menzies-Gow A, Phipps S et al. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. J Clin Invest 2003; 112:1029–36.
18 Phipps S, Benyahia F, Ou TT, Barkans J, Robinson DS, Kay AB. Acute allergen-induced airway remodeling in atopic asthma. Am J Respir Cell Mol Biol 2004; 31:626–32.
19 Lloyd CM, Gonzalo JA, Coyle AJ, Gutierrez-Ramos JC. Mouse models of allergic airway disease. Adv Immunol 2001; 77:263–95.
20 Lloyd CM, Gutierrez-Ramos JC. Animal models to study chemokine receptor function: in vivo mouse models of allergic airway inflammation. Methods Mol Biol 2004; 239:199–210.
21 Leigh R, Ellis R, Wattie J et al. Dysfunction and remodeling of the mouse airway persist after resolution of acute allergen-induced airway inflammation. Am J Respir Cell Mol Biol 2002; 27:526–35.
22 Leigh R, Ellis R, Wattie JN et al. Type 2 cytokines in the pathogenesis of sustained airway dysfunction and airway remodeling in mice. Am J Respir Crit Care Med 2004; 169:860–7.
23 Leigh R, Ellis R, Wattie J, Donaldson DD, Inman MD. Is interleukin-13 critical in maintaining airway hyperresposiveness in allergen-challenged mice? Am J Respir Crit Care Med 2004; 170: 851–6.
24 Leigh R, Southam DS, Ellis R et al. T-cell-mediated inflammation does not contribute to the maintenance of airway dysfunction in mice. J Appl Physiol 2004; 97:2258–65.
25 McMillan SJ, Lloyd CM. Prolonged allergen challenge in mice leads to persistent airway remodelling. Clin Exp Allergy 2004; 34:497–507.
26 Johnson JR, Wiley RE, Fattouh R et al. Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling. Am J Respir Crit Care Med 2004; 169:378–85.
27 Cho JY, Miller M, Baek KJ et al. Inhibition of airway remodeling in IL-5-deficient mice. J Clin Invest 2004; 113:551–60.
28 Humbles AA, Lloyd CM, McMillan SJ et al. A critical role for eosinophils in allergic airways remodeling. Science 2004; 305: 1776–9.
29 Blyth DI, Wharton TF, Pedrick MS, Savage TJ, Sanjar S. Airway subepithelial fibrosis in a murine model of atopic asthma: suppression by dexamethasone or anti-interleukin-5 antibody. Am J Respir Cell Mol Biol 2000; 23:241–6.
30 Hogaboam CM, Blease K, Mehrad B et al. Chronic airway hyperreactivity, goblet cell hyperplasia, and peribronchial fibrosis during allergic airway disease induced by *Aspergillus fumigatus*. Am J Pathol 2000; 156:723–32.
31 Zhu Z, Homer RJ, Wang Z et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. J Clin Invest 1999; 103:779–88.
32 Temann UA, Geba GP, Rankin JA, Flavell RA. Expression of interleukin 9 in the lungs of transgenic mice causes airway inflammation, mast cell hyperplasia, and bronchial hyperresponsiveness. J Exp Med 1998; 188:1307–20.
33 Rankin JA, Picarella DE, Geba GP et al. Phenotypic and physiologic characterization of transgenic mice expressing interleukin 4 in the lung: lymphocytic and eosinophilic inflammation without airway hyperreactivity. Proc Natl Acad Sci USA 1996; 93:7821–5.
34 Lee JJ, McGarry MP, Farmer SC et al. Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma. J Exp Med 1997; 185:2143–56.
35 Tang W, Geba GP, Zheng T et al. Targeted expression of IL-11 in the murine airway causes lymphocytic inflammation, bronchial remodeling, and airways obstruction. J Clin Invest 1996; 98: 2845–53.
36 Shinigawa K, Kojima M. Mouse model of airway remodelling: strain differences. Am J Respir Crit Care Med 2003; 168:959–967.

37 Snibson KJ, Bischof RB, Slocombe RF, Meeusen EN. Airway remodelling and inflammation in sheep lungs after chronic airway challenge with house dust mite. Clin Exp Allergy 2005; 35:146–52.
38 Tran MU, Weir AJ, Fanucchi MV et al. Smooth muscle hypertrophy in distal airways of sensitized infant rhesus monkeys exposed to house dust mite allergen. Clin Exp Allergy 2004; 34:1627–33.
39 Phipps S, Benyahia F, Ou TT, Barkans J, Robinson DS, Kay AB. Acute allergen-induced airway remodeling in atopic asthma. Am J Respir Cell Mol Biol 2004; 31:626–32.

H. H. Kariyawasam and D. S. Robinson
*Allergy and Clinical Immunology*
*National Heart and Lung Institute and Leukocyte Biology Section*
*Biomedical Sciences Division*
*Faculty of Medicine*
*Imperial College London*
*Sir Alexander Fleming Building*
*Exhibition Road*
*London SW7 2AZ*
*UK*
*E-mail: d.s.robinson@imperial.ac.uk*