SUPPLEMENTARY METHODS

Characteristics of the Study Population

Virus infections could account for most symptomatic respiratory disease. [15] Twelve viruses (rhinoviruses, coxsackie viruses, echoviruses, enteroviruses, coronaviruses 229E and OC43, respiratory syncytial virus (RSV), influenza A and B, parainfluenza viruses 1-3 (PIF), adenoviruses, human metapneumovirus) and 2 bacteria (*Chlamydia pneumoniae* and *Mycoplasma pneumoniae*) were analysed from nasal aspirates by PCR following a reported respiratory infection in the first year. Rhinoviruses were the most common virus detected (48%), followed by RSV (10.9%), coronaviruses (5.8%), PIF (5.4%) and influenza (4.3%). [15]

44% (12/27) of the children with current asthma at 5 years did not have a history of asthma recorded for previous years. All of the SPT+ children who had doctor-diagnosed asthma at age 3 had asthma at age 4 and 5. In contrast 6/13 SPT- children who were diagnosed with asthma at 3 years of age did not present with asthma later. All the 3-year old asthmatics were followed up at age 4 but five asthmatic children (2 SPT+ and 3 SPT-) in the 4-year group were not followed up at age 5.

Antigen preparation

Natural Der p 1 was purified from spent mite medium by antibody affinity chromatography using the 4C1 monoclonal antibody. The P4 and P6 outer membrane proteins of *H. influenzae* from the Eagen isolate and Der p 2.0101 were produced as fusion polypeptides with N-terminal hexa-histidine tags in pQE-80L (Novagen, Madison, USA) for P4 and P6 and pET-11d (Novagen) for Der p 2. PspA1 (family 1, clade 2) was derived from the pneumococcal strain Rx1 (aa 1-302), PspA2 (family 2, clade 3) from the V-24 strain (aa 1-410) and PspC (clade B) from the D39 strain (aa 1-445). The Psp proteins were cloned as fusion proteins with a C-terminal six-histidine tag in pET20b (Novagen). The pQE-80L, pET-11d and pET20b-based constructs were expressed in BL21 Star (DE3) pLysS (Novagen) using 1 mM isopropyl-b-D-thiogalactopyranoside (IPTG), in the presence of 100 µg/ml ampicillin and 34 µg/ml chloramphenicol (Invitrogen Corp., Carlsbad, USA). The majority of the expressed recombinant proteins were purified under non-denaturing conditions using Ni^{2+}-nitrilotriacetic acid (Ni-NTA) agarose chromatography (Qiagen GmbH, Germany), according to the manufacturer's protocols. Der p 2.0101 was produced under denaturing conditions and refolded prior to Ni-NTA purification. Fractions containing the relevant proteins were pooled, dialyzed into 10 mM Tris-HCl, pH 7.4, 100 mM NaCl, 2 mMEDTA, and applied to a Bio-Rad Macro-Prep cation (Der p 2, P4, PspC) or anion (P6, PspA1, PspA2) exchange support (Bio-Rad, Hercules, USA). Elution was achieved with a linear gradient of 100 to 500 mM NaCl in Tris-HCl, pH 7.4, 2 mM EDTA. Fractions containing the relevant protein were pooled and further purified using size exclusion chromatography by applying the samples to either a HiPrep 26/60 Sephacryl HR 100 (Der p 1, Der p 2, P4 and P6) or HR S200 (PspA1, PspA2, PspC) column (GE Healthcare Life Sciences, Buckinghamshire, UK). A single peak was obtained for each of the proteins. Finally, the proteins were sterilized and endotoxin removed using 0.2-µm Mustang E filters (Pall Life Sciences, Portsmouth, UK). The purities of all the proteins were checked on a 12.5% sodium dodecyl sulfate-polyacrylamide gel and the concentrations determined using the optical density at 280 nm (OD280) measurements and extinction coefficients.