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Bronchial reactivity to histamine and bradykinin is unchanged after rhinovirus infection in normal subjects

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Bronchial reactivity to histamine and bradykinin is unchanged after rhinovirus infection in normal subjects. Q.A. Summers, P.G. Higgins, I.G. Barrow, D.A.J. Tyrrell, S.T. Holgate.

ABSTRACT: We investigate the effects of rhinovirus (RV) infection on airways reactivity.

Twenty seven normal volunteers (11 atopic) were inoculated with RV 2 or RV EL. The provocative concentrations of histamine and bradykinin required to produce a 15% fall in the forced expiratory volume in one second (FEV_1) ($PC_{15}FEV_1$) were measured before, 7 and 21 days after inoculation. Infection was determined by a fourfold rise in anti-viral antibody titre and by viral culture from nasal washings. Peak expiratory flow rate (PEF) was recorded three days before and for 21 days after inoculation.

All subjects underwent the first two bronchial challenges, and 22 the third challenge. For the whole group and for atopic subjects, there were significant correlations between the PC_{15} values for bradykinin and histamine (r=0.82 and r=0.85, respectively). Twenty subjects were infected; six had clinical colds. For the 16 infected subjects who had all three challenges, the median (range) $PC_{15}FEV_1$ for the histamine challenges was 36 (0.89–64), 62 (1.5–64) and 34 (0.94–64) $mg \cdot ml^{-1}$, respectively, and 32 $mg \cdot ml^{-1}$ for each bradykinin challenge (range: 0.015–32, 0.088–32 and 0.033–32). There were no significant differences between study days for $PC_{15}FEV_1$ histamine or bradykinin for the whole group, the infected subjects, those with clinical colds or for those infected with either RV subtype. There was no significant change in mean daily PEF after viral infection.

We conclude that airways reactivity to histamine and bradykinin is unchanged after experimental RV infection in normal volunteers.

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Respiratory tract infection by respiratory syncytial viruses, rhinovirus (RV), influenza A and B, and parainfluenza 3 can precipitate asthma attacks in children and adults [1, 2]. How such infections induce asthma is unknown, although infection with influenza and RV can damage the bronchial epithelium [3] and produce destruction and dysfunction of ciliated nasal epithelium, respectively [4, 5]. There are over 100 serotypes of RV [6], of which 90% (the major group) bind to a single receptor found on many different human cells, the intercellular adhesion molecule 1 (ICAM-1) [7]. The minor group of RV serotypes bind to a second receptor, which has yet to be clearly characterized. The expression of ICAM-1 may be upregulated in the bronchial epithelium of allergen-sensitized and challenged primates [8], although whether this occurs in asthma is not known.

Although when infected with respiratory viruses, many normal adults experience symptoms of chest tightness and bronchial irritability, the evidence to support an increase in nonspecific bronchial reactivity following either community-acquired or experimental RV infection is not strong. EMPEY *et al.* [9] were first to report such an increase, and one other study has reported increased airways reactivity after infection with RV 16 [10]. Others have failed to show any increase in airways reactivity after RV infection in either normal non-atopic or atopic volunteers [11–13], or in patients with asthma [13, 14]. In assessing bronchial reactivity, these studies have used agonists which mainly effect airway narrowing through stimulation of specific receptors coupled to the contraction of airways smooth muscle [15, 16]. On the basis that RV infection may damage or interfere with the function of the epithelium, stimuli revealing neurogenic aspects of bronchial reactivity might be more appropriate.

In an attempt to clarify the relationship between experimental RV infection and subsequent changes in airway reactivity, we have examined the effects of such an infection in normal volunteers on the bronchial response to inhaled histamine and

bradykinin. The latter stimulus is considered to represent bronchoconstriction mediated by sensory nerve stimulation [17].

Methods

Twenty seven normal subjects were recruited from subjects volunteering for studies at the Medical Research Council (MRC) Common Cold Unit (CCU) in Salisbury, UK. Twelve were male and the mean age (SD) of the group was 39 (8.3) yrs. Seven subjects were current smokers and 11 were atopic on the basis of a clinical history of atopy (allergic rhinitis, eczema) and/or positive skin prick tests to one or more of six common aeroallergens. None had prior or current asthma and all had baseline forced expiratory volume in one second (FEV_1) values >70% of predicted. Subjects gave written informed consent. Following a general clinical examination and withdrawal of blood for anti-viral antibody measurements, subjects were placed in quarantine for 10 days. On day 3, approximately 100 50% tissue culture infectious doses $(TCID)_{50}$ of RV 2 (minor group) and RV EL (major group) were instilled separately into the nasal passages of each volunteer [18].

Before and after inoculation, until discharge from the CCU, symptoms and signs were scored on a daily basis by a standardized procedure [19]. Each volunteer was given a clinical grade (nil, very mild, mild, moderate, severe), based on the observer's overall assessment of the volunteer's response. A close correlation exists between an individual's symptom score and the clinical grade allocated to that subject [20]. Subjects recorded and scored on a 4-point scale the presence of cough, dyspnoea, wheeze and chest tightness, for day and night, before and for three weeks after inoculation. On days 3 and 6–10, nasal washings were collected for virus detection by inoculating tissue cultures of Helen Lake (HeLa) tumour cells [21]. On day 21 after inoculation, a convalescent blood sample was obtained, and antibody titres to the two RV strains assayed in the acute and convalescent sera by neutralization tests in HeLa cells [21].

Pulmonary function was recorded as the FEV_1 on a wedge bellows spirometer (Vitalograph, Bucks, UK), and as PEF using a mini-Wright peak flow meter. Whilst at the CCU, subjects recorded the best of three PEF readings every 3 h during waking hours. After discharge from the CCU, PEF was recorded twice daily on rising and retiring until 21 days after inoculation.

Bronchial challenge with histamine and bradykinin was performed using a modified Chai technique [22, 23]. Histamine challenge was undertaken at least 2 h before the bradykinin challenge. The challenge was terminated when the FEV_1 fell by ≥15% or when the highest dose of agonist had been inhaled. The provocative concentration producing a 15% fall in FEV_1 ($PC_{15}FEV_1$) was derived by linear interpolation, or by extrapolation if the FEV_1 fell by ≥10% but <15% after the highest dose. If after the highest dose of agonist (32 $mg \cdot ml^{-1}$ for histamine, 16 $mg \cdot ml^{-1}$ for bradykinin) the fall in FEV_1 was <10%, an estimated (or censored) value of 64 or 32 $mg \cdot ml^{-1}$, respectively, was assigned as the PC_{15} representing the next doubling concentration of agonist [24]. Challenges were undertaken on day 2, and at 7 and 21 days following virus inoculation.

Analyses of data

Data are presented as the mean (SEM) unless otherwise stated. Subjects were regarded as infected if there was virus shedding (as detected by viral culture from the nasal washings) or a ≥fourfold rise in viral antibody titres between the acute and convalescent sera. In addition, a clinical response was considered to be present on the basis of the clinical grading allocated by the observer. The effects of virus infection on histamine and bradykinin PC_{15} between study days were compared for those subjects who had all three bronchial challenges. Because some of the PC_{15} data are censored, the group results for these are presented as the median (range). PC_{15} values for the atopic and non-atopic subjects for the two challenges were compared by the Mann-Whitney U test. Friedman's two-way analysis of variance was used to compare changes in the PC_{15} values between study days for all subjects, and the following subgroups: infected subjects, atopics, infected atopic subjects, those with clinical colds and those subjects infected with each virus serotype. The relationship between histamine and bradykinin responsiveness was examined by Kendall's coefficient of concordance. The mean PEF for each subject was calculated for each day, and logarithmically transformed. To assess the effect of virus inoculation the mean daily PEF for the three days up to and including the inoculation day were taken to represent baseline, and a comparison was made between this value and the subsequent mean daily PEF values by one-factor analysis of variance (ANOVA). For this analysis, only subjects who had completed PEF recordings for the whole three week period were included.

Results

All subjects underwent the first and second bronchial challenge procedures, while 22 were able to attend for the third bronchial challenge, the remainder being unable to do so for social reasons. There was no significant difference between the mean age of the infected and non-infected subjects, 38.7 (1.9) *vs* 38.3 (2.8) yrs (Student's paired t-test). The atopic subjects were significantly younger than the non-atopic subjects, 34.2 (2.6) *vs* 41.6 (1.6) yrs; p=0.027.

For the whole group of 27 subjects, the median preinoculation PC_{15} values for histamine and bradykinin were 44.3 (0.89–64) and 32 (0.015–32) $mg \cdot ml^{-1}$, respectively. Prior to virus inoculation, PC_{15}

values <32 mg·ml^{-1} for histamine and <16 mg·ml^{-1} for bradykinin were obtained in six out of the nine atopics and in three and two of the 11 non-atopics, respectively. There were significant differences in PC_{15} values between the atopic and non-atopic subjects for the initial histamine challenge (10.05 (0.9–64) *vs* 64 (13.9–64) mg·ml^{-1}, p=0.0019) and for the initial bradykinin challenge (4.6 (0.015–32) *vs* 32 (0.2–32) mg·ml^{-1},p=0.0068). There was also a significant correlation between the initial PC_{15} values for bradykinin and histamine for the whole group (r=0.82, p<0.0001), for the atopic subjects (r=0.85, p<0.0001), and for the non-atopic subjects (r=0.68, p=0.003).

being 35.5 (0.9–64), 62.3 (1.5–64) and 34 (0.9–64) mg·ml^{-1}, respectively. Although the atopic subjects were more reactive to histamine at baseline, at 7 and 21 days after inoculation there were no significant changes. When those with "clinical" colds were analysed separately, there were no significant differences between the PC_{15} values derived from the successive histamine challenges. Similarly, there were no significant differences in PC_{15} histamine when the following subgroups were analysed: infected subjects who did not have clinical colds, infected patients who were not atopic, subjects infected with RV 2, and those infected with RV EL. In those with proven virus infection or

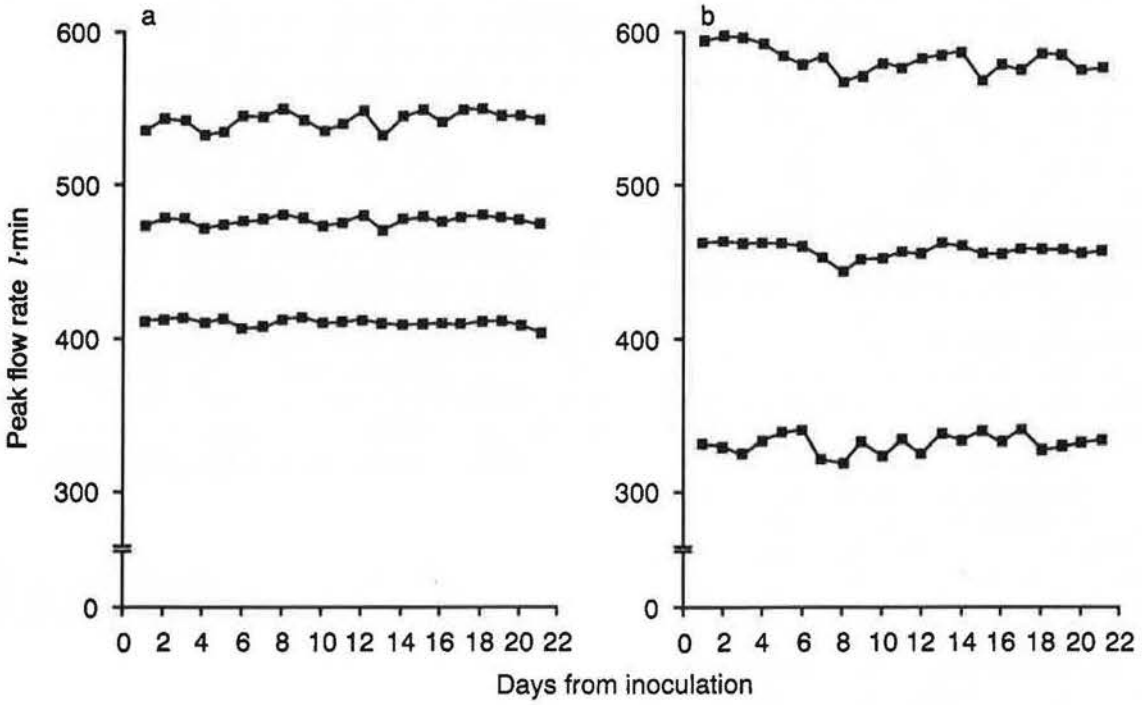

Fig. 1. – Mean daily peak flow rate with 95% confidence intervals for: a) subjects infected with rhinovirus (RV) RV 2; and b) subjects infected with RV EL.

Twenty (74%) subjects were infected by one or more of the criteria detailed above. Seventeen of the 27 subjects responded with a fourfold or greater rise in antibody titre to one or other of the two virus strains, and virus was isolated from nasal washings in 17 of the infected subjects. Six subjects developed unequivocal "clinical" colds and five further subjects milder symptoms not amounting to a definite cold. All subjects considered to have a "clinical cold" were shown to be infected by seroconversion, the presence of virus in nasal washings, or both.

In the 16 infected subjects who completed all three challenges, there was no significant change in the PC_{15} histamine between baseline and day 7 after inoculation, between baseline and day 21 after inoculation, or between 7 and 21 days after inoculation, the median (range) PC_{15} values for the three histamine challenges

those with "clinical" colds no significant differences were found for PC_{15} bradykinin between baseline and measurements at 7 and 21 days postinoculation, or between the latter time points, whether analysed for the whole group or for those who were atopic.

After virus inoculation, there was no significant change in the mean daily PEF values for the whole group or for the atopic subjects, the non-atopic subjects, those with colds, those subjects considered infected and for those subjects infected with either strain of RV. Figure 1 shows the mean daily PEF for subgroups infected with each RV subtype. Similarly, there was no change in any of the symptom scores for dyspnoea, wheeze, chest tightness or cough. Indeed, only three subjects recorded such symptoms after infection, and these were short-lived (less than three days) mild cough.

Discussion

After nasal inoculation of 27 non-asthmatic subjects with two strains of RV, there was laboratory evidence of viral infection in 20 and, of these, a definite symptomatic cold was seen in six, with equivocal symptoms in five. For the whole group, the group of infected subjects and all of the subgroups, there were no statistically significant changes in airways reactivity to inhaled histamine or bradykinin at any time point.

Airways response was expressed as the PC_{15} FEV_1 because none of the subjects were asthmatic, and because this value corresponds well with the PC_{20} [25]. In attempting to explain why the two strains of RV failed to cause an increase in bronchial responsiveness, it may be that we failed to induce an infection of sufficient severity, even though 55% developed a "clinical" cold. Although HALPERIN *et al.* [12] have pointed out that approximately 25% of viral respiratory tract infections are clinically inapparent, and it is known that a direct relationship exists between the clinical severity of RV infection and the occurrence of lower airway symptoms in asthmatic subjects [26], the observations made in the present study represent the clinical picture of RV infections as they commonly occur. Ten of the initial 27 volunteers failed to develop neutralizing antibody against the relevant virus and to induce a more severe clinical response we should have had to give an inappropriately high dose of virus or administer it to the lower airways by aerosol. It may also be possible that the postinoculation challenges were performed too long after inoculation to detect changes in airways reactivity, as LEMANSKE *et al.* [10] reported changes when challenges were done three days after inoculation. However, the timing of exacerbations of asthma or increases in airways reactivity related to RV infection are not known, and it was considered that any clinically relevant changes should have been detectable.

Comparisons between the various studies of airways reactivity after RV infections are hampered by the large differences in the design and reporting of the different studies. The most important differences between the studies that preclude direct comparisons are the RV strains used and the wide range of instilled doses. There is little information regarding the pathogenicity of the various RV subtypes, or the magnitude of the inoculum needed to produce a symptomatic (as opposed to subclinical) response. However, because these differently designed studies were predominantly negative, the weight of evidence is against RV-induced increases in airways reactivity, particularly as two of the studies had asthmatics in their study population [12, 13]. Nor is it likely that the dose of virus is an important factor as the study in which the highest dose of virus was used did not show any increase in airways reactivity [11], in contrast to the study of LEMANSKE *et al.* [10] in which the same RV strain was used at only a slightly lower dose. Moreover, the inhalation challenges used in the different studies were performed and reported in a non-standardized manner, introducing further difficulties in making direct comparisons between studies.

Thus, there was no support for our hypothesis that RV-induced epithelial disruption would be reflected by an enhanced airways response to bradykinin. The lack of response to both bradykinin and histamine, in combination with the predominantly negative experimental studies quoted above, does not support the view that RV infection may be associated with changes in airways reactivity [27], despite the results of epidemiological studies which demonstrate the association of RV infection with exacerbations of asthma. Our findings are compatible with those of JOSEPHS *et al.* [28] who found that natural exacerbations of asthma can occur without concomitant changes in bronchial reactivity, even in the presence of an upper respiratory tract infection.

In conclusion, we have shown that in a group of atopic and non-atopic volunteers experimental RV infection produced no change in airways reactivity to inhaled histamine or bradykinin 1 and 3 weeks after infection. On the basis of this and other studies, we feel that the evidence for an increase in airways reactivity following experimental RV infection is not strong, and suggests that the clinical impression of wheezing being exacerbated or induced by RV infection takes place through mechanisms other than those responsible for altering airways reactivity. Our data do not, however, preclude the induction or exacerbation of asthma by other infectious agents.

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