

Inhalant allergen-specific T-cell reactivity is detectable in close to 100% of atopic and normal individuals: covert responses are unmasked by serum-free medium

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Summary

Background It is widely held that *in vitro* T cell responses to allergens are more prominent in atopic than in normal individuals, though this conclusion is based upon culture techniques which fail to detect proliferative responses in a significant minority of atopics and many normals.

Objectives: Study allergen-specific proliferative responses of T cells cultured in serum-free medium (SFM). Examine associations between atopic status, age and T cell reactivity.

Methods Initially, peripheral blood mononuclear cells were stimulated with allergens or antigens in SFM, and compared with cells cultured in RPMI + 10% fetal calf serum or human AB serum. Subsequently, T cell reactivity was studied in 34 adults (20–49 years), 27 children (2–13 years), and 19 infants (≤ 10 weeks) using SFM alone.

Results Compared with serum-supplemented medium, SFM enhanced net T cell proliferation, both in bulk culture and when cloning at limiting dilution. In many subjects, SFM unmasked T cell reactivity to allergens which was not otherwise evident, and lowered the threshold allergen levels required for *in vitro* T cell triggering. For most allergens, T cell proliferative responses did not differ between adults who had specific IgE, and those who did not. The most vigorous responses observed were to ubiquitous inhalant allergens, which stimulated T cells from close to 100% of adults and children, and over 60% of infants. In contrast, responses to the 'vaccine' antigen tetanus toxoid were completely absent in the latter age group, but present in the majority of adults and children.

Conclusions These findings suggest that the extent of active T cell recognition of environmental allergens has been hitherto underestimated, and further that these responses may frequently be initiated in very early life. Additionally, these findings reinforce the notion that qualitative (as opposed to quantitative) variations in specific T cell reactivity ultimately determine allergen responder phenotype.

Keywords: T-cell reactivity, proliferative response, atopics, serum-free medium

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Introduction

IgE synthesis in response to environmental allergens is a major feature of atopy. Although it has been clearly established that IgE production is T cell regulated, the basis for the distinction at the T cell level between normal and allergic individuals remains only partially understood. *In vitro* proliferative responses of T cells to allergens have been examined over the past two decades, with the general consensus being that while it is not possible to routinely detect reactivity in all allergic individuals [1,2], their responses are more frequent and of greater magnitude in allergic than in normal individuals [1–7]. Moreover, some authors have reported a virtual absence of allergen-specific T cell proliferation in normal subjects [1,2]. Proponents of the alternative view, that T cell proliferative responses to allergens are of similar prevalence in allergics and normals, have been in the minority [8,9]. However, this view is supported by two published studies on allergen-specific T cell cloning frequency [10,11], and by the finding that allergen-specific serum IgG antibodies are detectable in virtually all adults and children [12,13], implying underlying T helper cell activity.

A more recent and now widely accepted view is that the key to the expression of allergic disease lies in the pattern of cytokine production by allergen-specific T helper cells, as epitomised by the skewed TH-2-like profile which characterizes allergen-specific clones from atopic individuals [14–16]. One important corollary of this model is that allergen-reactive T cells should be readily demonstrable in both normal and allergic individuals, which is seemingly at odds with many of the observations referred to above [1–7].

It is possible that these, and other related contradictions in the literature, reflect the magnitude of interlaboratory variation in the performance of human peripheral blood T cells in superficially identical *in vitro* assay systems. This is explained in the folklore of the field as T cell culture being 'more of an art than a science'. However, it is likely that a significant proportion of this variability is due to covert methodological problems, associated with the composition of the nutrient solutions employed to support T cell growth. The latter comprise two major components: chemically formulated commercial growth media, which are carefully standardized by the manufacturers, and serum supplements that are almost universally accepted as obligatory for optimal T cell growth, and which vary widely between batches and from laboratory to laboratory. Serum from all mammalian species contains a complex, and highly variable mixture of foreign antigens and biological response modifiers which can influence *in*

vitro T cell function. Platelet derived growth factor in serum, for example, has been shown to selectively inhibit synthesis of some T cell lymphokines, including T cell growth factors [17]. Serum supplements in T cell culture medium also commonly trigger high 'background' proliferation, such that the specific component of T cell responses can be obscured. The compromise solution to this problem which is widely practised in immunology laboratories, involves prescreening of pooled serum for acceptable batches based on the *in vitro* performance of 'reference' T cells. However, this process is *ad hoc*, and must inevitably contribute to interlaboratory variability.

In attempts to circumvent this problem, some laboratories have developed their own lymphocyte growth media which do not require serum supplementation, with varying success [18]. Recently, serum free medium (SFM) from several commercial sources has become available. These are generally based on existing formulations of salts and amino acids etc, but are supplemented with standardized amounts of selected serum components such as albumin, peptide hormones, transferrin and fatty acids. Based on initial screening, we selected one such SFM for more detailed studies, comprising a cross-sectional survey of T cell reactivity to a panel of environmental allergens, in adults and children. We report below that the use of SFM results in a substantial and unequivocal improvement in the sensitivity of allergen-specific T cell activation assays, relative to those performed in conventional medium with human or fetal calf serum (FCS) supplements. In particular, SFM facilitates detection of large T cell responses to ubiquitous environmental allergens such as house dust mite in virtually all normal (as well as atopic) individuals, and unmasks similar responses in infants which are detectable only sporadically in conventional culture systems.

The increased sensitivity of this procedure, and its inherent capacity for interlaboratory standardization, suggests that it should prove a useful tool for elucidation of some of the unresolved issues relating to allergen-specific T cell reactivity in normals vs atopics.

Materials and methods

Study population

Thirty-four adults (16 male, 18 female; age range 20–49 years) were recruited from community newspaper advertisements and from laboratory staff. The study took place in autumn, outside the local pollen season. After informed consent, serum was collected for measurement of specific IgE to dust mite, rye pollen, cat, egg white and

milk, using the Pharmacia CAPS system. (A RAST score of ≥ 2 on a scale of 1–6 was regarded as positive.) Twelve subjects had no symptoms of allergic disease, were radioallergosorbent assay (RAST) negative to all allergens, and had a mean ($\pm$ SD) total IgE of 31 ± 45 KU/ml. Ten persons had either no symptoms, or trivial symptoms of allergic rhinitis (not requiring treatment), a positive RAST test to at least one allergen, and a mean total IgE of 84 ± 121 KU/mL. A further 12 individuals had symptomatic asthma or allergic rhinitis which required treatment, a positive RAST test to at least one allergen, and a mean total IgE of 250 ± 150 KU/mL. No subjects had received specific immunotherapy, nor had they been treated with oral corticosteroids within the preceding 3 months.

For comparison, we also studied 19 infants (age ≤ 10 weeks) and 27 children (2–13 years) who were part of a longitudinal community based study. All had at least one first degree relative with allergic disease, and were therefore at increased risk for the development of subsequent allergy themselves. Blood was obtained following informed parental consent. The study was approved by the local institutional ethics committee.

Culture medium

Except where indicated, cells were cultured in serum-free medium (AIM-V lymphocyte medium, Gibco, New York, USA). Information from the manufacturer indicates that this preparation contains DMEM and F12 media, supplemented with unspecified amounts of human albumin, insulin, transferrin and lipids, together with HEPES buffer, L-glutamine, streptomycin and gentamicin. To this, we added 2-mercaptoethanol (4×10^{-5} M; Sigma) in-house. In some experiments, direct comparison was made with conventional serum-containing medium, using RPMI (Gibco), supplemented with 10% heat-inactivated serum, either pooled human AB serum or fetal calf serum (FCS), together with L-glutamine, gentamicin and 2-mercaptoethanol.

Antigens

Whole house dust mite extract was the kind gift of Dr G. Stewart (Perth), and was prepared using *Dermatophagoides pteronyssinus* obtained from CSL Ltd (Melbourne, Australia). Native Der p 1 and Der p 2 were purified by affinity chromatography from spent mite medium as previously described [19]. Whole rye extract was also from Dr G. Stewart, and affinity purified Lol p 1 was obtained from Professor B. Knox (University of Melbourne, Australia). Ovalbumin was purchased from Sigma, and tetanus toxoid and bovine serum albumin

(BSA) were from CSL Ltd, BSA was used as it contains many allergens which are present in cow's milk, and we included tetanus toxoid as a control antigen. Preliminary experiments had determined that maximal T cell proliferation was induced by the following concentrations of allergens/antigens: whole mite 30 μ g/mL, whole rye 50 μ g/mL, Der p 1 and 2 30 μ g/mL, Lol p 1 10 μ g/mL, ovalbumin 100 μ g/mL, BSA 100 μ g/mL and tetanus toxoid 0.3 Lf/mL (data not shown).

Cell preparation and proliferation assays

Blood was collected and diluted with an equal volume of RPMI containing preservative free heparin. Peripheral blood mononuclear cells (PBMC) were isolated on a density gradient (Lymphoprep, Nycomed, Norway), washed three times in RPMI containing 2% FCS, and re-suspended in culture medium. PBMC (2×10^5 /well) were cultured in replicates of five in 96-well round-bottom trays in a volume of 200 μ L, using concentrations of antigen as described above. Parallel control wells contained no antigen. Following incubation at 37°C in 5% CO₂ for 6 days, DNA synthesis was measured by adding ³H thymidine (0.5 μ Ci; Amersham). Cultures were harvested onto glassfibre filter mats 16–18 h later, and thymidine uptake assayed as counts per minute (cpm) using a Packard Matrix 9600 beta counter. Results were subsequently converted to dpm via an appropriate standard curve, and expressed as either of the following: Delta dpm = median of wells containing antigen minus median of control wells. Stimulation ratio = median of wells containing antigen $\div$ median of control wells.

A delta dpm of > 2500 , together with a stimulation ratio (SR) ≥ 3.0 was taken as a positive response, these being fairly conservative criteria. Data were expressed as mean $\pm$ SD; except where indicated, comparisons of the magnitude of proliferative responses were analysed by the Mann–Whitney *U*-test for unpaired samples. The proportions of positive responses in different subject groups were compared and analysed using Fisher's exact test; two-sided *P* values are reported. A *P* value of < 0.05 was regarded as statistically significant.

Limiting dilution analysis

The precursor frequency of house dust mite specific T cells was determined by limiting dilution analysis. Serum-free medium was compared with AB supplemented RPMI in parallel experiments. T cells were purified from PBMC by dual cycle rosetting using neuraminidase treated sheep erythrocytes as described elsewhere [20]. Varying numbers of T cells (10^4 , 5000,

2500, 1250 and 0 cells/well) were cultured in 20 μ L medium in 60-well Terasaki plates together with whole mite extract (30 μ g/mL) and 10^4 irradiated (3000 rad) autologous non-T cells. For each dilution of T cells, 24 wells were set up with antigen; 12 wells without antigen served as controls. Tritiated thymidine was added on day 6, and uptake measured 16–18 h later.

In these experiments, replicate counts at each cell concentration showed a skewed distribution (though with an approximately constant coefficient of variation), so the data was $\log_e$ transformed prior to analysis [21]. Antigen stimulated wells were regarded as positive if the thymidine uptake was greater than the $\log_e$ mean + 2 SD of the corresponding wells without antigen. Precursor frequencies and 95% confidence limits were obtained in a standard manner [21] using generalized linear modelling. The proportion of negatively responding wells in each plate was treated as a binomially distributed response variable, and the $\log_e$ of the corresponding cell concentration per well was used as a regression offset [21].

Having invoked a complementary log-log link, the regression constant (α) then provided a direct estimate of the precursor frequency (i.e. exponential α) and 95% confidence intervals for this frequency were obtained as exponential ($\alpha \pm 1.96 \times s$) where 's' was the estimated standard error of α [21].

Results

Influence of medium on T cell proliferation

Our laboratory has been studying *in vitro* responses of human T cells to allergens over the last decade, employing various human AB serum pools as growth supplements, and our findings collectively have been in agreement with those in references [1–7] cited above. The responses obtained below in human AB-supplemented medium are consistent with our former experience with some of the same allergen preparations [2,7].

In initial experiments in the current series, we compared the ability of serum-supplemented and SFM to support polyclonal T-cell responses to recall antigens (Fig. 1a). High background thymidine incorporation was observed in FCS containing cultures, obscuring any significant antigen specific component of the responses. Lower backgrounds were present in cultures containing AB serum and in SFM. Analysis of the data derived from cultures in AB and SFM revealed that while significant responses to tetanus toxoid were obtained in both systems, responses to mite were significant only in the SFM cultures (SFM: $P = 0.005$ vs background; AB:

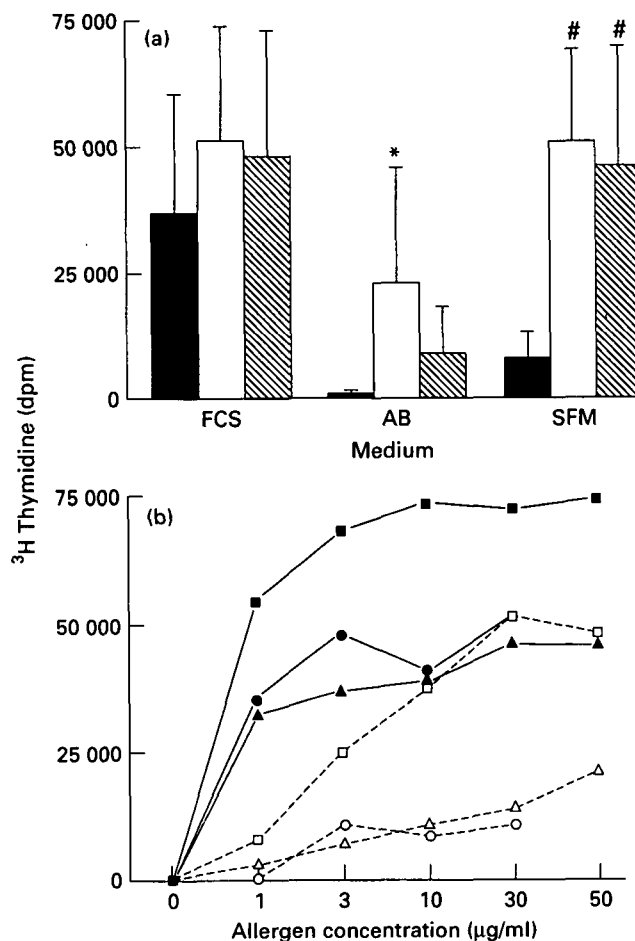


Fig. 1. Influence of culture medium on T cell responses to antigens/allergens. In parallel experiments, PBMC were cultured in either serum free medium (SFM), or RPMI supplemented with FCS or human AB serum. (a) Polyclonal proliferative responses to tetanus toxoid or whole mite extract, as determined by ^3H thymidine uptake, are shown as dpm. Data represent mean $\pm$ SD of eight experiments. Statistical significance of differences between stimulated and background (control) wells was analysed by paired *t*-test. *indicates $P < 0.05$ vs background; # indicates $P < 0.01$ vs background; unmarked columns indicate $P > 0.05$ vs background. ■. Background. □. Tet tox. ▨. Mite. (b) Representative dose response to mite allergens in one individual. Employing the criteria described in Materials and Methods, all responses shown were positive with the exception of Der p 1 and Der p 2 in AB.

SFM AB
 —■— Mite ---□---
 —▲— Der p 1 ---△---
 —●— Der p 2 ---○---

Table 1. Comparison of precursor frequencies of house dust mite specific T cells cultured in SFM, and RPMI supplemented with human AB serum, as determined by limiting dilution analysis. Numbers in brackets refer to 95% confidence intervals. In experiments 2, 3 and 4, background responses in serum containing medium were high, such that it was not possible to quantify precursor frequency in a statistically acceptable manner. Experiments 1 and 2 were from normal subjects, and experiments 3 and 4 from mite-allergic subjects

Experiment	Medium	Precursor frequency (95% CI)
# 1	SFM	1/2026 (1/1482–1/2771)
	AB	1/14910 (1/8543–1/26022)
# 2	SFM	1/3121 (1/2250–1/4330)
	AB	Not possible to quantify
# 3	SFM	1/1798 (1/1315–1/2459)
	AB	Not possible to quantify
# 4	SFM	1/5799 (1/3948–1/8516)
	AB	Not possible to quantify

$P = 0.09$ vs background). Furthermore, the largest net responses (Δ dpm) to antigen or allergen occurred with SFM.

Dose titration experiments in five adults using whole mite, Der p 1 and Der p 2 showed that T cell proliferation in serum free medium was either superior, or equivalent to that in AB/RPMI. In every subject, SFM reduced the threshold concentration required to elicit proliferation to at least one of these three allergens (an example is shown in Fig. 1b). For some allergens, otherwise covert T-cell reactivity was unmasked by the use of SFM (e.g. responses to Der p 1 and Der p 2 in Fig. 1b).

Thus, SFM appeared superior to AB/RPMI for bulk cultures of antigen stimulated T cells. However, it was not clear whether SFM would support the growth of T cells at low densities in culture, and hence be useful in the isolation and propagation of allergen-specific clones. When we compared the ability of SFM and AB/RPMI to estimate the precursor frequency of mite specific T cells using limiting dilution analysis, SFM also appeared preferable to AB/RPMI. The precursor frequencies of mite-specific T-cells are shown in Table 1. In three of the four experiments, it was not possible to quantify precursor frequency of cells cultured in AB/RPMI because the background thymidine uptake of wells without antigen overlapped that of wells containing antigen.

T-cell proliferation to different allergens

Having established that the use of SFM increased both

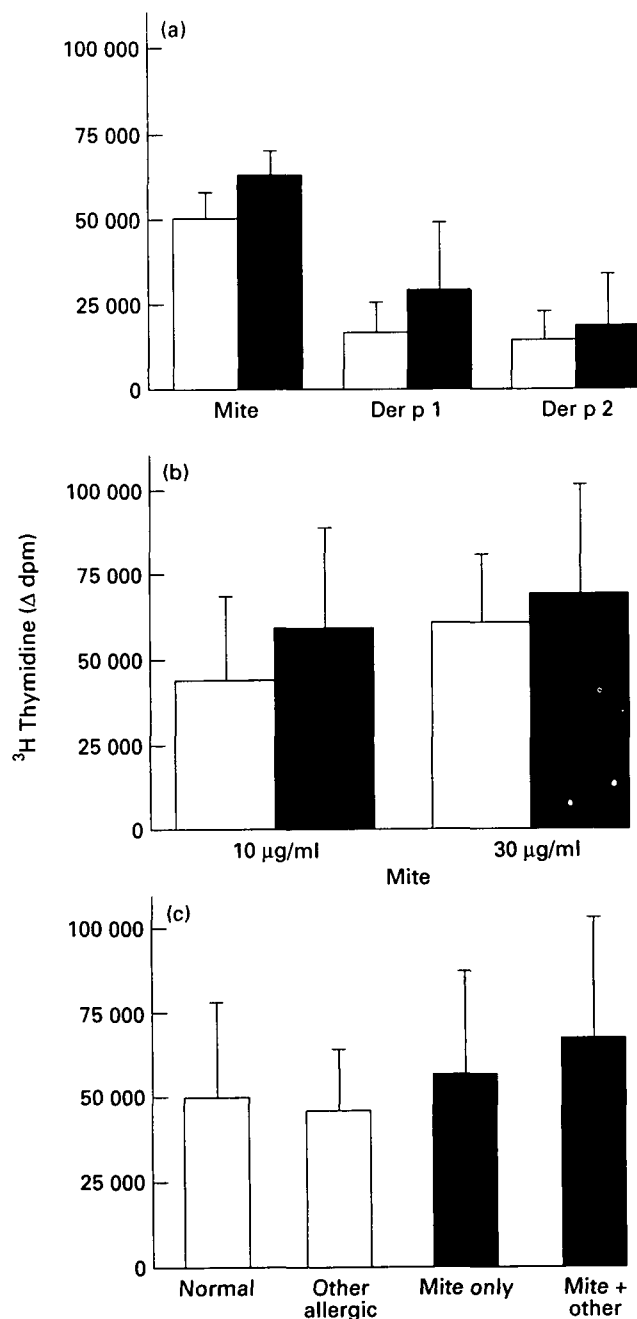


Fig. 2. T cell proliferation in response to mite allergens in subjects with ($n = 18$), and without ($n = 16$), mite specific IgE. For each allergen, proliferation did not differ significantly between subject groups. Data is expressed as mean $\pm$ SD; backgrounds have been subtracted. (a) Maximal responses to optimal concentrations of whole mite, Der p 1 and Der p 2. (b) Proliferation at two concentrations of whole mite. (c) Maximal proliferation to whole mite in normal subjects ($n = 12$), those with specific IgE directed to other allergens ($n = 4$), those with mite allergy only ($n = 6$), and those with mite plus other allergies ($n = 12$). There were no significant differences between the four groups by ANOVA ($P = 0.49$). Mite-specific IgE: $\square$. Absent. $\blacksquare$. Present.

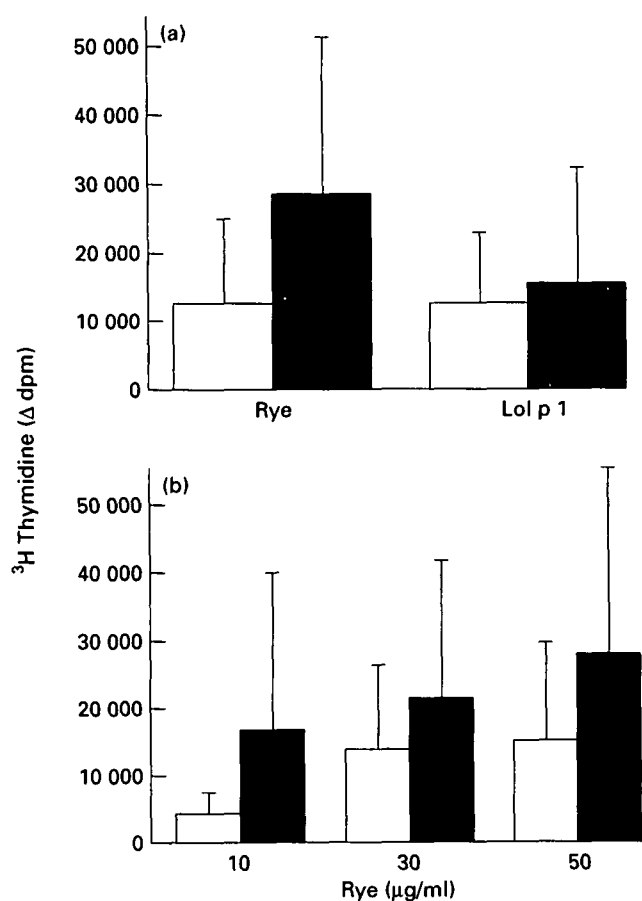


Fig. 3. T cell proliferation in response to rye allergens in subjects with ($n = 12$) and without ($n = 22$) rye-specific IgE. Data is expressed as mean $\pm$ SD; backgrounds have been subtracted. (a) Maximal proliferation in response to optimal concentrations of whole rye and Lol p 1. For whole rye, but not Lol p 1, proliferation was significantly greater in those with rye-specific IgE; $P < 0.01$. (b) Proliferation at three concentrations of whole rye. Proliferation was significantly greater in those with rye-specific IgE only at 10 $\mu\text{g/ml}$; $P < 0.05$. Rye-specific IgE: $\square$. Absent. $\blacksquare$. Present.

the ability to detect *in vitro* antigen-specific T cell reactivity, and the magnitude of responses obtained, we then studied T cell proliferation to a panel of recall allergens/antigens in 34 adults. With respect to the mite allergens, there was no difference in the magnitude of maximal proliferative responses between those who had mite-specific IgE, and those who did not (Fig. 2a). For whole mite extract this also held true at the two concentrations of mite extract studied (Fig. 2b). Even when the atopic subjects were subdivided into those who were purely mite-allergic and those also sensitized to other allergens, no differences in the magnitude of T cell proliferation was observed (Fig. 2c). Likewise, there

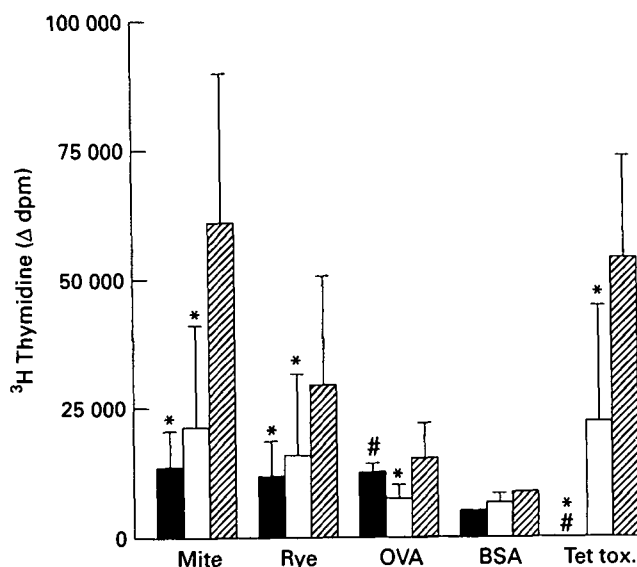


Fig. 4. T cell proliferative responses to allergens/antigens in infants ≤ 10 weeks, in children aged 2–13 years, and in adults aged 20–49 years. Data represent the magnitude of ^3H thymidine uptake (mean $\pm$ SD) in those with positive responses (where Δ dpm > 2500 , and stimulation ratio ≥ 3). * $P < 0.05$ compared with adults; # $P < 0.05$ compared with children. $\blacksquare$. Infants. $\square$. Children. $\hatched$. Adults.

was no significant association between the absolute level of mite-specific IgE and the size of the proliferative responses to whole mite ($r = 0.15$, $P > 0.4$; Pearson correlation; data not shown). Using the criteria defined in Materials and Methods, positive proliferative responses to either whole mite, Der p 1 or Der p 2 were detected in 100% of mite sensitized and 94% of normal individuals ($P = 0.47$, Fisher's exact test).

The magnitude of T cell proliferation induced by whole rye appeared to be greater in those with rye-specific IgE than in those without (Fig. 3a), particularly at the lowest dose of allergen studied (10 $\mu\text{g/ml}$; Fig. 3b). However, a parallel difference in proliferative responses to the major allergen Lol p 1 was not found (Fig. 3a). Moreover, positive responses to either whole rye or Lol p 1 were observed in 50% of rye allergic subjects, and also in 50% of those without rye allergy.

Only two adults had specific IgE directed to egg or milk. As shown in Table 2 and Fig. 4, T cell proliferation in response to these antigens was infrequent and of small magnitude, and appeared unrelated to the presence of specific IgE.

Inter-age comparison of T cell proliferation

When T cell responses in adults were compared with

Table 2. Detection of T-cell proliferative responses in infants, children and adults. Data are expressed as the percentage of positive responders, as defined by Δ dpm > 2500, and a stimulation ratio (SR) ≥ 2 , or ≥ 3 . For each allergen, the statistical significance of differences in response rates between age groups were assessed using Fisher's exact test. * $P < 0.05$ compared with adults; # $P < 0.05$ compared with children. All infant and children's T cell samples responded positively to stimulation with anti-CD3 (data not shown)

	SR ≥ 2			SR ≥ 3		
	Infants	Children	Adults	Infants	Children	Adults
Mite	74 *	89	100	63 *	89	91
Der p1	—	—	100	—	—	89
Der p2	—	—	95	—	—	74
Mite or Der p1 or p2	—	—	100	—	—	97
Rye	33 *	61	71	28 #	61	38
Lol p1	—	41	62	—	37	26
Rye or Lol p1	—	68	82	—	68	50
OVA	35	38	44	29	35	18
BSA	29	33	26	7	29 *	3
Tet tox	0 * #	57	73	0 * #	57	56

those in infants and children a number of trends emerged. Firstly, mite responses were prominent at all ages, whether the data was expressed as the percentage of positive responders (Table 2), or as the magnitude of proliferation in those responders (Fig. 4). Significant T cell proliferative responses to mite allergens were present in close to 100% of adults and children. Even in infants less than 10 weeks of age, mite-specific responses were observed in over 60%.

Secondly, in adults, T cell proliferative responses to food allergens (ovalbumin and BSA) were generally smaller and less frequent than those due to the inhalants (whole mite and rye), whereas such differences were not obvious in infants. For tetanus toxoid, T cell proliferation was absent in all infants, none of whom had been immunized. All the children and adults had a history of prior tetanus immunization; tetanus-specific T cell responses were seen in at least 50%.

Discussion

The principal findings to emerge from this study of *in vitro* allergen-specific T cell responses can be summarized as follows. SFM enhanced allergen-specific T cell proliferation (compared with standard serum supplemented medium), and in many subjects unmasked reactivity to allergens which was not otherwise evident. Proliferative responses to ubiquitous inhalant allergens could be detected in nearly all adults, regardless of their atopic

status. Furthermore, the magnitude of these T cell responses did not differ in most cases between those individuals who had specific IgE, and those who did not. Finally, distinct age-related differences were noted in the pattern of T cell responses to individual allergens.

The particular SFM studied herein appears to overcome many of the difficulties encountered with the use of serum supplemented medium. T cell proliferative responses to antigens were generally improved, both in bulk culture and at limiting dilution, and otherwise covert T-cell reactivity was unmasked in many subjects.

Under these conditions of optimized T cell growth, the magnitude of maximal allergen-specific proliferative responses did not generally differ between adults who had specific IgE to the particular allergens tested, and those adults who did not. The findings for whole rye were the exception, though it should be noted that proliferative responses to the major allergen Lol p 1 were similar in rye allergic and non-rye allergic subjects (Fig. 3a). Though the observations in Fig. 3b provide some evidence that the threshold dose of allergen required for T-cell triggering may be lower in allergen-sensitized individuals than in normal individuals, this conclusion remains speculative until a wider range of allergen concentrations are studied in a large number of individuals, using comprehensive dose-response protocols such as those in Fig. 1b. There was no evidence that the method of analysing T-cell proliferation influenced the conclusions of the study: whether thymidine

incorporation was expressed as Δ dpm or as stimulation ratios, our overall conclusions remained the same.

It is noteworthy that T cell proliferation in response to mite allergens could be detected in close to 100% of adults, and the size of the responses was larger than for any other allergen, implying a high frequency of mite-specific T cells in the circulation. Though it has previously been suggested that crude mite extracts may be mitogenic [1], this suggestion seems unlikely given that responses to the purified major allergens Der p 1 and Der p 2 were similarly high. The lower frequency of proliferative responses to rye allergens may be due to the seasonal nature of this allergen, and to the fact that the study was conducted outside the local pollen season, whereas dust-mite allergen is ubiquitous in the local environment throughout the year.

The conclusion that the magnitude of proliferative responses to optimal stimulatory concentrations of allergen do not differ between allergen-sensitized and normal individuals is contrary to reports from a number of investigators [1–7], and we speculate that allergen-specific T cell responses in normal individuals may be particularly susceptible to the inhibitory effects of factor(s) in serum. It is feasible that quantitative differences in T cell proliferative responses may exist between atopic and normal subjects, but demonstrating such differences may require the use of individuals with more severe allergic disease than those employed in this study. Even though the lack of a consistent association between levels of specific IgE and the magnitude of allergen-specific T cell proliferation argues against the preceding proposition, this is clearly a subject which should be addressed further using SFM. Nonetheless, our findings reinforce the current view that the key to the distinction between allergic and normal immune responses lies in qualitative (rather than quantitative) variations in T cell reactivity, such as those exemplified by the proposed human equivalent of the murine TH-1/TH-2 paradigm [16].

The use of SFM also facilitated detection of allergen-specific T cell reactivity in young children, in particular responses to whole mite, rye and ovalbumin, which were observed in a large proportion of infants less than 10 weeks of age. It is highly likely that these represent a genuine evolving T cell memory response, and are not artefacts of the culture medium employed, as responses to tetanus toxoid were absent in this age group who had not yet been immunized. This improved T cell culture system is likely to be of particular benefit in studies of primary T cell sensitization in infants, which rely on the capacity to detect allergen-specific T cells, which theoretically will be present at extremely low precursor frequencies. Recent studies from several groups have

reported low-grade T cell proliferative responses to allergens in cord blood and infant blood samples [22–25]. Our results (as shown in Table 2 and Fig. 4), together with ongoing experiments with cord blood which are demonstrating high levels of allergen-specific T cell proliferation, suggest that the use of SFM may also be a useful adjunct to these important studies.

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