

Human T-cell responses to *Trichophyton tonsurans*: inhibition using the serum free medium Aim V

J. B. SLUNT*, E. A. TAKETOMI*† and T. A. E. PLATTS-MILLS*

*Asthma and Allergic Center, Department of Internal Medicine, University of Virginia Health Sciences Center, Charlottesville, VA, USA and †Immunology Division, Department of Pathology, Federal University of Uberlandia, Uberlandia, Minas Gerais, Brazil

Summary

Background Proteins of the fungus *Trichophyton tonsurans* have been shown to give strong T cell proliferative responses *in vitro* using lymphocytes from individuals with immediate or delayed skin tests. Furthermore, *Trichophyton*-specific T-cell lines produce distinct patterns of cytokine production depending upon the skin-test reactivity of the host. However, skin-test negative individuals generally give limited responses. A recent study has demonstrated dust mite specific proliferation with lymphocytes from atopic and non-atopic subjects using the serum free medium Aim V.

Objective Compare the T-cell reactivity to *Trichophyton* and dust mite antigens in Aim V and media containing 10% pooled AB serum.

Methods Peripheral blood mononuclear cells (PBMC) were separated from subjects with different skin-test reactivities and cultured either in media with serum or serum free media.

Results Proliferation to two *Trichophyton* extracts was decreased in serum free media among subjects with either immediate or delayed hypersensitivity. *Trichophyton* skin-test negative subjects gave poor proliferative responses in both culture conditions. A similar decrease in proliferation in serum free media was observed in cultures with PHA and tetanus toxoid. In contrast, the majority of individuals showed increased proliferation to dust mite antigens when cultured in serum free media. Cultures in serum free medium produced less IFN γ , IL-4, or IL-5 compared with cultures in AB medium.

Conclusions *In vitro* T-cell responses to the dermatophyte fungus *T. tonsurans* are inhibited by the serum free medium Aim V. This inhibition is seen equally with cells from individuals with delayed and immediate hypersensitivity. Furthermore, the results do not support the view that AB serum is masking T-cell responses in skin-test negative individuals.

Keywords: T lymphocytes, *Trichophyton tonsurans*, allergy, delayed type hypersensitivity, Aim V serum free media

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Introduction

Immediate hypersensitivity (IH) is characterized by T-cell responses of the TH₂ phenotype with the production of IL-4 and IL-5, while delayed hypersensitivity (DH) is thought to be mediated by TH₁ cells characteristically producing IFN γ

[1–7]. Cells of the TH₀ phenotype produce cytokines characteristic of both TH₁ and TH₂ cells [6–8]. TH₁ and TH₂ cells negatively interact with each other; IFN γ inhibits the development of TH₂ cells, while IL-4, at high doses, suppresses the development of TH₁ cells [9–11]. These two distinct immune responses have been demonstrated by skin-test reactivity and cytokine production to protein IV, a purified protein from the dermatophyte fungus *Trichophyton tonsurans* [12]. *Trichophyton* can induce an immediate hypersensitivity (IH) skin-test reaction, which is positive

Correspondence: Dr T. Platts-Mills, Asthma and Allergic Diseases Center, Box 225, University of Virginia Health Sciences Center, Charlottesville, VA, 22908, USA.

within 15 min, and has been associated with IgG4 and IgE antibodies and a TH₀/TH₂ like T-cell cytokine profile *in vitro* [13–16]. Alternatively, delayed hypersensitivity (DH) is also common among subjects with a history of athlete's foot disease and is characterized by an indurated skin-test reaction at 24–48 h [17–20]. This response has been associated with IgG antibodies and a TH₁ cytokine profile *in vitro* [16]. In addition, there are individuals who are skin-test negative to *Trichophyton*.

It is important to understand the *in vitro* factors that can play a role in the development of TH₁ and TH₂ cells. Serum is commonly added to lymphocyte cultures and is a major source of inter-laboratory variation. In recent years, a number of commercial suppliers have developed 'serum free media' that contain defined amounts of a limited number of serum components. Recently, Upham *et al.* [21] reported almost 100% of non-atopic subjects had a peripheral blood mononuclear cell (PBMC) proliferative response to dust mite allergens at a level similar to that seen with atopic individuals using the serum free medium Aim V. This result was surprising in that a number of laboratories have reported that non-allergic subjects have limited or no allergen specific proliferative responses [22–24]. The authors speculate that there may be inhibitory factors in serum which normally mask the proliferative response of non-atopic individuals, and that under proper conditions, the difference between non-atopic and allergic T-cell responses may be qualitative (TH₁ vs TH₂) rather than quantitative (proliferation) [21].

In order to examine the response to *Trichophyton*, we have cultured cells from subjects with IH, DH, and negative skin-test reactivities in both media containing 10% human AB serum (AB media) and in Aim V serum free media (SFM). In addition, we have studied the proliferative response in both types of media to tetanus toxoid, phytohemagglutinin (PHA), and dust mite antigens in dust mite skin-test positive (IH) and negative subjects. Our results show an increase in the level of proliferation to dust mite antigens in SFM, however, the response to both *Trichophyton* and tetanus toxoid was decreased. Decreased proliferation in SFM was seen equally in subjects with immediate and those with delayed hypersensitivity skin-test reactions.

Materials and methods

Cell culture media

Aim V (Life Technologies, Gaithersburg, MD, USA) is a prepared HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) buffered media composed of equal parts of Dulbecco's Modified Eagle Medium and Ham's Nutrient Mixture F-12 with purified human albumin, transferrin, insulin, lipids, L-glutamine, streptomycin, and

gentamicin [21,26]. 2-mercaptoethanol (0.05 mM) (Life Technologies) was added to this media [21]. AB media consisted of 10% pooled human AB serum (Sigma, St Louis, MO, USA), penicillin (100 U/mL), streptomycin (100 µg/mL), MEM (minimal essential medium) amino acids solution (1×), MEM non-essential amino acids solution (0.1 mM), sodium pyruvate (1.0 mM), L-glutamine (2 mM), HEPES buffer (15 mM) (Life Technologies, Gaithersburg, MD, USA), and 50 µg/mL gentamicin solution (Sigma) in RPMI-1640 (BioWhittaker, Walkersville, MD, USA).

Skin testing

Study subjects with a history of athlete's foot disease were recruited by an advertisement. Dust mite allergic subjects were recruited from laboratory personnel. Individuals were prick skin tested with extracts of the house dust mite *Dermatophagoides pteronyssinus* (Dpt) and *Trichophyton* mix (1:10 w/v), which contains *T. tonsurans*, *T. rubrum*, and *T. mentagrophytes* species (Hollister-Steir, Miles, Spokane, WA, USA) [12,15]. Test sites were examined at 15 min and a positive IH skin test was defined by a weal ≥ 6 × 6 mm for both extracts. If the skin-prick test to *Trichophyton* was negative, 0.02 mL of allergen (1:200 w/v) was given by intradermal injection. Tests sites were again examined after 15 min, and at 24 and 48 h. A positive DH reaction was distinguished by an induration ≥ 6 mm in diameter at 24 or 48 h. Two of the eight subjects with a positive IH skin-test reaction to *Trichophyton* had evidence of asthma attributed to fungal sensitivity, and five of the thirteen dust mite allergic subjects had evidence of disease attributed to dust mite sensitivity.

Allergen preparation

Tetanus toxoid (Massachusetts Public Health Biologic Laboratories, Jamaica Plain, MA, USA) was used at a final concentration of 10 µg/mL. Two fractions of *Trichophyton tonsurans* (T. tons.), 0–60% (8.0 mg/mL) and 60–90% (3.1 mg/mL) were prepared from a crude mycelial extract by precipitation with saturated ammonium sulfate. The 0–60% T. tons. extract was used at a final concentration of 40 µg/mL while the 60–90% T. tons. extract was used at 15.5 µg/mL. Dpt extract was prepared from dried material kindly provided by Dr Larry Arlian (Wright State University, Dayton, Ohio, USA), contained 285 µg/mL Der p 1 and 121 µg/mL Der p 2, and was used at a final concentration of 1.0 µg/mL. Recombinant (r) Der p 2 was expressed in bacteria and used at a final concentration of 10 µg/mL. Phytohemagglutinin (Sigma) was used at 5 µg/mL.

PBMC proliferation assays

Blood (60–120 mL) was taken into syringes containing preservative free heparin (Sigma) (10 U/mL blood) and diluted 1:2 in RPMI-1640. Venipuncture was approved by the Human Investigations Committee at the University of Virginia. Peripheral blood mononuclear cells were isolated by density gradient centrifugation over Ficoll-Paque (Pharmacia, Uppsala, Sweden). Cells (3×10^5 /well) were cultured at 37°C in triplicate in 96 well plates (Sarstedt, Newton, NC, USA) with antigens in the appropriate medium. For the final 16 h, the cells were incubated in the presence of ^3H -thymidine (ICN, Irvine, CA, USA) before being harvested onto a glass fibre filter and counted with a Packard (Meriden, CT, USA) Matrix 9600 direct beta counter. Results are expressed as the change in counts per minute (Δcpm) and/or as a stimulation index (SI), calculated by dividing the mean of triplicate wells with antigen by the mean of triplicate wells cultured without antigen. A stimulation index ≥ 5 was defined a positive proliferative response. Allergen concentrations used were those found to be optimal for PBMC proliferation at day 7 in pilot dose response studies.

Cytokine assays

Freshly isolated PBMC were cultured with allergen extracts (Dpt or T. tons.) in a 24 well plate (2×10^6 cells/well). On day six 30 U/mL of recombinant IL-2 (Hoffman LaRoche, Nutley, NJ, USA) was added. Cell culture supernatants were harvested at various times from separate wells, and cytokines were assayed using ELISA assays (IL-4 and IL-5, Pharmingen, San Diego, CA, USA) (IFN γ Endogen, Cambridge, MA, USA). Results are expressed in ng/mL, as compared with a standard curve generated from recombinant cytokines. The lower level of detection for the cytokine assays ranged from 0.10 to 0.30 ng/mL for the IFN γ assay, 0.10 to 0.30 ng/mL for the IL-4 assay, and 0.10 to 0.75 ng/mL for the IL-5 assay. Allergen concentrations used for cytokine production analysis were those found to be optimal for PBMC proliferation which may not be optimal for cytokine production.

Statistical analysis

Statistical significance was assessed using a two-tailed distribution paired Student's *t*-test.

Results

Cultures in SFM were directly compared with parallel cultures in AB media. The proliferative response to the two *Trichophyton* extracts, 0–60% T. tons. and 60–90% T.

tons., was decreased in SFM as compared with AB media among all three skin-test groups at day 7 (Fig. 1). The decrease among DH subjects was statistically significant using both allergens ($P < 0.001$), while the decrease among the IH subjects was significant only to the 0–60% T. tons. extract ($P < 0.05$). Table 1 shows the mean results expressed in both counts per minute above background (Δcpm) and stimulation index at days 5 and 7. The PBMC

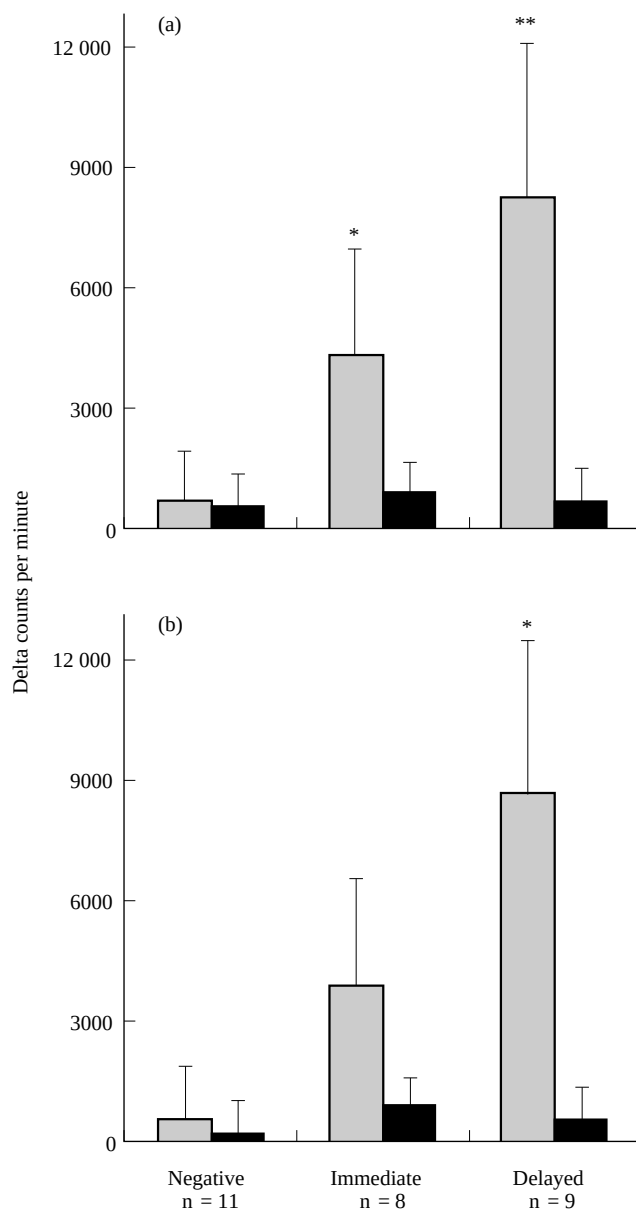


Fig. 1. Mean level of proliferation to *Trichophyton* extracts and standard deviations at day 7 among the three *Trichophyton* skin test groups in AB media $\square$ and SFM $\blacksquare$. (a) 0–60% T. tons. extract, (b) 60–90% T. tons. extract. (* $P < 0.05$, ** $P < 0.001$ as compared with SFM).

Table 1. Mean levels and standard deviations of *Trichophyton* specific proliferation in AB media and SFM. Results expressed in counts per minute above background (Δ cpm) and stimulation indices

Skin test	Harvest day	n	0–60% T. tons. extract				60–90% T. tons. extract			
			AB media		SFM		AB media		SFM	
			Δ cpm	SI	Δ cpm	SI	Δ cpm	SI	Δ cpm	SI
Negative	5	9	217 $\pm$ 295	2.4 $\pm$ 1.9	277 $\pm$ 509	2.3 $\pm$ 1.6	125 $\pm$ 129	1.8 $\pm$ 0.7	–5 $\pm$ 329	1.4 $\pm$ 0.6
	7	11	635 $\pm$ 1295	3.7 $\pm$ 2.7	510 $\pm$ 833	6.8 $\pm$ 13	570 $\pm$ 867	4.5 $\pm$ 4.6	179 $\pm$ 341	1.9 $\pm$ 0.7
IH	5	8	1731 $\pm$ 2527	19 $\pm$ 30	978 $\pm$ 539	4.7 $\pm$ 4.1	1722 $\pm$ 1710	18 $\pm$ 20	507 $\pm$ 380	2.7 $\pm$ 1.4
	7	8	4300 $\pm$ 2700	20 $\pm$ 13	926 $\pm$ 720	9.2 $\pm$ 11	3864 $\pm$ 5160	17 $\pm$ 16	862 $\pm$ 594	5.5 $\pm$ 5.7
DH	5	8	3100 $\pm$ 2299	16 $\pm$ 10	838 $\pm$ 729	3.8 $\pm$ 2.3	3344 $\pm$ 2862	17 $\pm$ 12	469 $\pm$ 319	2.7 $\pm$ 1.5
	7	9	8269 $\pm$ 3888	54 $\pm$ 72	642 $\pm$ 891	6.0 $\pm$ 8.2	8625 $\pm$ 5228	62 $\pm$ 83	452 $\pm$ 759	5.4 $\pm$ 6.9

proliferative responses were higher at day 7 than at day 5 and at both time points the DH group had the highest level of proliferation.

The proliferative responses to PHA and tetanus toxoid in AB media and SFM were compared at days 5 and 7. There was a significant decrease in the level of proliferation to tetanus toxoid at both day 5 and 7, and to PHA at day 5 using SFM (Fig. 2). The proliferative response to the mitogen PHA was maximal at day 5 in both AB media and SFM, while the antigen specific tetanus toxoid response was highest at day 7.

A time course study was done with tetanus toxoid and the 60–90% T. tons. extract in a subject who had a DH skin-test response to *Trichophyton*. The time course to both antigens peaked at day 7 and the cultures in SFM showed very little response at any time from day 5 to day 12 (Fig. 3).

The proliferative response among dust mite skin-test negative and positive (IH) individuals was measured to dust mite extract and the purified recombinant dust mite allergen Der p 2. At day 5 and day 7, there was a statistically significant ($P < 0.05$) increase in the mean level of Δ cpm among skin-test negative subjects in SFM as compared with AB media with Dpt extract. Also the mean level of both the Δ cpm and stimulation index were increased with rDer p 2 (Table 2). There were also increases in both the Δ cpm and stimulation indices among the dust mite IH subjects in the SFM. Comparing individual subjects at day 5, 9/12 skin-test negative individuals had an increase to Dpt extract as did 11/12 to rDer p 2 (Fig. 4A). The proliferative response was increased in SFM to both Dpt extract and rDer p 2 among all 10 IH subjects (Fig. 4B). Lymphocytes cultured in SFM induced similar levels of proliferation in both atopics and non-atopics on days 5 and 7 (data not shown).

Supernatants were collected from cultures of PBMC obtained from skin-test negative subjects (Table 3). Cells from subjects KA and ET cultured with Dpt extract in SFM

produced IFN γ , and a similar cytokine pattern was observed in AB media with subject ET. In contrast, cultures from three subjects (AM, PH, and KM) exhibited a clear difference in the amount of IFN γ and IL-5 produced in the two types of media, with higher levels of both cytokines being detected in the wells with AB media. Cells from skin-test positive individuals (IH and DH) were also cultured in SFM and AB media (Table 4). All subjects except SS produced high levels of cytokines (>1 ng/mL of IL-4, IL-5, or IFN γ) after stimulation with either the 60–90% T. tons. extract or Dpt extract. Little to no cytokines were detected in the supernatants of parallel cultures established in SFM. Overall the results demonstrate poor production of IFN γ , IL-4, and IL-5 in cultures with serum free medium.

Discussion

It is uncertain what factors affect the development of TH₁ and TH₂ cells, but the following have been suggested: different antigen presenting cells, cytokines present during the initial presentation of antigen, the nature of the antigen, the dose of the antigen, and the route of exposure [6,7,9,10,26–31]. In light of recent data, it is also unclear whether nonallergic individuals can produce specific T-cell proliferative responses [21]. Our study has focused on the dermatophyte fungus *Trichophyton tonsurans* because it is different from most other allergens in that it can induce both IH and DH skin-test responses. The goal of the present study was to determine whether SFM could increase the proliferative response to *Trichophyton* antigens among skin-test negative subjects and whether SFM would have different effects on the IH and DH proliferative response.

The results showed a significant decrease in proliferation to *Trichophyton* proteins among IH and DH subjects in SFM. Among the skin-test negative subjects, this decrease was much lower however SFM did not unmask proliferative

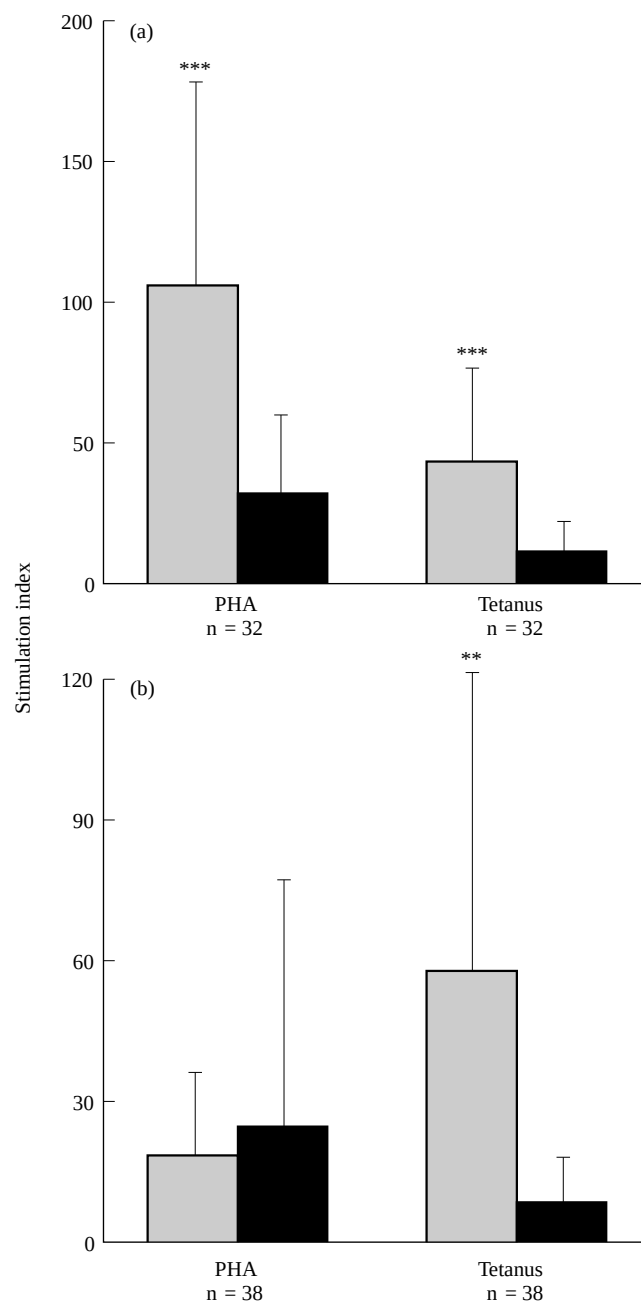


Fig. 2. Mean level of PBMC proliferation and standard deviations to PHA and tetanus toxoid in AB media $\square$ and SFM $\blacksquare$. (a) Day 5 harvest, (b) Day 7 harvest (** $P < 0.001$, *** $P < 0.0001$ as compared with SFM).

responses. The *Trichophyton* extracts induced a very low mean proliferative response (cpm < 1100) among this subject group in AB media, which is in keeping with previous data [16]. Among the other subject groups the level of proliferation was higher using AB media (>4000 cpm) and in these groups the decrease in SFM was clear.

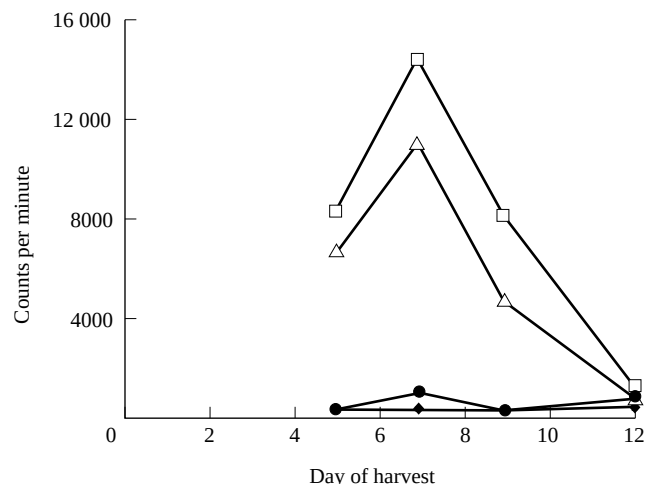


Fig. 3. PBMC proliferative response time course of a subject with a DH skin test to *Trichophyton*. Tetanus toxoid and T. tons. extract cultured in AB media and SFM. $\square$, Tetanus toxoid in AB media; $\triangle$, T. tons. in AB media; $\bullet$, Tetanus toxoid in SFM; $\blacklozenge$, T. tons. in SFM.

Our PBMC proliferation results to dust mite antigens in SFM are similar to those reported by Upham *et al.* [21]. In that manuscript they reported that SFM caused an increase in proliferation to dust mite and rye grass allergens among non-atopic subjects. While they did not report on the proliferative response to PHA or *Trichophyton* in SFM, their manuscript did report that the tetanus toxoid proliferative response was unaffected. However, the authors stated that only 56% of the 34 adults they tested had a stimulation index ≥ 3 in response to tetanus toxoid in SFM at day 7. This is a low threshold for a positive response, and it would be interesting to know what percentage of subjects would have had the same level of proliferation in AB media. Our results for tetanus toxoid in SFM were similar, 20/38 subjects (52.6%) had a SI ≥ 3 , however in AB media, 37/38 (97.4%) of the subjects had a SI ≥ 3 .

Cultures of cells from skin-test negative subjects in SFM with either T. tons. extract or Dpt extract produced low levels of cytokines as compared with parallel cultures in AB media. Skin-test positive subjects (IH and DH) also did not produce cytokines in SFM despite the fact that in AB media high levels of IL-4, IL-5 and IFN γ have been demonstrated. The lack of cytokine production in response to *T. tonsurans* in SFM is not surprising since proliferation to this antigen is strongly inhibited in this medium. However, no cytokines were produced by cells from dust mite allergic IH subjects cultured with Dpt extract in SFM. By contrast, cells from two out of four skin-test negative subjects produced IFN γ in response to Dpt extract in SFM. Therefore, the increase in proliferation observed with SFM is not necessarily correlated with IFN γ production.

Table 2. Mean levels and standard deviations of dust mite specific proliferation in AB media and SFM. Results expressed in counts per minute above background (Δ cpm) and stimulation indices

Skin test	Harvest day	n	Dpt extract				rDer p 2			
			AB media		SFM		AB media		SFM	
			Δ cpm	SI	Δ cpm	SI	Δ cpm	SI	Δ cpm	SI
Negative	5	12	66 $\pm$ 141	1.9 $\pm$ 1.2	527 $\pm$ 429	3.2 $\pm$ 1.8	737 $\pm$ 1006	3.8 $\pm$ 2.1	1900 $\pm$ 3243	6.3 $\pm$ 6.1
	7	14	104 $\pm$ 653	2.0 $\pm$ 1.1	658 $\pm$ 517	5.9 $\pm$ 8.4	1281 $\pm$ 1435	5.2 $\pm$ 5.4	1521 $\pm$ 1809	10.4 $\pm$ 11.4
IH	5	10	359 $\pm$ 518	3.6 $\pm$ 3.7	890 $\pm$ 948	4.3 $\pm$ 4.0	475 $\pm$ 517	4.1 $\pm$ 3.5	1225 $\pm$ 758	5.6 $\pm$ 3.5
	7	13	944 $\pm$ 1209	6.2 $\pm$ 8.0	662 $\pm$ 446	7.5 $\pm$ 6.9	2313 $\pm$ 1711	11 $\pm$ 9.8	2009 $\pm$ 1711	14 $\pm$ 15

It is interesting to note that Upham *et al.* [21] reported that the use of SFM did not result in increased proliferation in response to all the allergens they investigated. They report that positive responses to either whole rye or Lol p 1 were seen in only 50% of rye allergic subjects as well as 50% of those without rye allergy. In addition, among 34 adults studied (two with IgE to either milk or egg), only 3%

had a positive proliferative response ($SI \geq 3$) to bovine serum albumin, while only 18% had a positive response to ovalbumin. It is unclear whether these low values are due to SFM causing a decrease in the proliferative response, as we have demonstrated with *Trichophyton* and tetanus, or whether these allergens induce a poor proliferative response in any type of media.

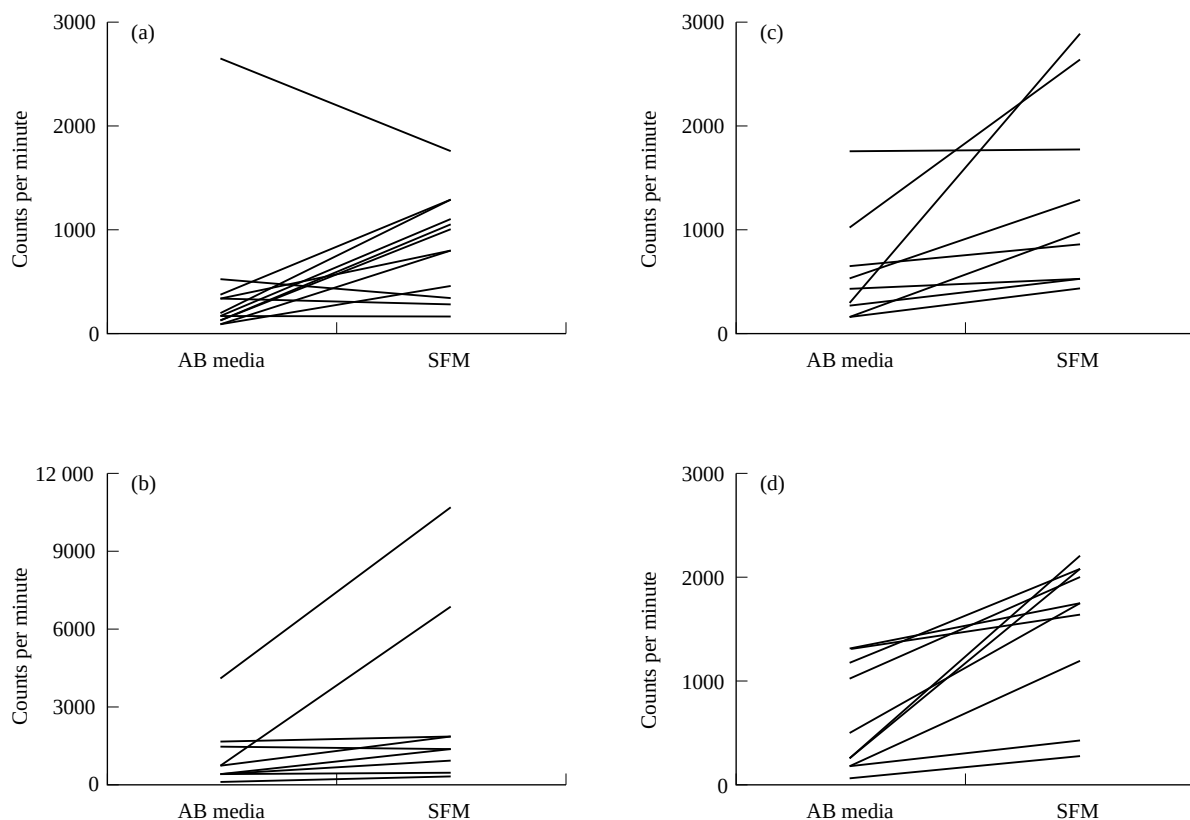
**Fig. 4.** Effect of SFM on PBMC proliferative response to dust mite antigens as compared with AB media at day 5. (a) Skin-test negative subjects to Dpt extract and (b) skin-test negative subjects to rDer p 2; (c) immediate hypersensitivity subjects to Dpt extract and (d) immediate hypersensitivity subjects to rDer p 2.

Table 3. Cytokine production (ng/mL) by PBMC from skin-test negative individuals in AB and SFM media at various days post stimulation with antigen

Subject	Antigen	Media	Day 7			Day 10			Day 13		
			IFN γ	IL-4	IL-5	IFN γ	IL-4	IL-5	IFN γ	IL-4	IL-5
KA	Dpt	SFM	1.5	<0.2	<0.1	>5.0	<0.2	<0.1	2.7	0.47	<0.1
ET	Dpt	AB	<0.1	<0.1	<0.1	3.7	0.52	<0.1	4.1	<0.1	<0.1
		SFM	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	5.0	0.11	<0.1
AM	Dpt	AB	0.40	<0.1	0.54	>5.0	<0.1	2.0	>5.0	<0.1	1.3
		SFM	<0.1	<0.1	<0.1	0.32	<0.1	<0.1	0.28	<0.1	<0.1
PH	Dpt	AB	—	—	—	<0.1	<0.1	0.32	4.2	0.10	2.2
		SFM	—	—	—	<0.1	<0.1	<0.15	<0.1	<0.1	<0.15
	T. tons.	AB	—	—	—	0.25	0.64	8.4	<0.1	<0.1	7.2
		SFM	—	—	—	<0.1	<0.1	<0.15	<0.1	<0.1	<0.15
KM	T. tons.	AB	<0.1	<0.1	<0.75	0.43	<0.1	2.0	<0.1	<0.1	1.3
		SFM	<0.1	<0.1	<0.75	<0.1	<0.1	<0.75	<0.1	<0.1	<0.75

It is not clear why SFM selectively enhances the proliferative response to dust mite allergens. It is interesting to note that both our study and the previous work using SFM found a high percentage of subjects with a positive proliferative response with inhaled allergens and a lower response with allergens that are not inhaled [21]. They reported poor proliferative response to food allergens,

which are believed to be absorbed through the gut, and the present study showed low proliferative responses with *Trichophyton tonsurans*, an organism that grows on the outer surface of the body and is believed to be absorbed through the skin [12,15,32,33]. Tetanus is another antigen that is not inhaled; being introduced to the immune system by injection. It is therefore possible that the difference

Table 4. Cytokine production (ng/mL) by PBMC from skin-test positive (IH or DH) individuals in AB and SFM media at various days post stimulation with antigen

Subject	Skin test	Antigen	Media	Day 7			Day 10			Day 13		
				IFN γ	IL-4	IL-5	IFN γ	IL-4	IL-5	IFN γ	IL-4	IL-5
AA	IH	Dpt	AB	—	—	—	1.1	0.36	3.2	0.82	<0.1	4.5
			SFM	—	—	—	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	DH	T. tons.	AB	—	—	—	0.15	<0.1	2.2	<0.1	<0.1	2.2
			SFM	—	—	—	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
SS	IH	Dpt	AB	<0.1	<0.2	<0.1	<0.1	<0.2	<0.1	0.16	<0.2	<0.1
			SFM	<0.1	<0.2	<0.1	<0.1	<0.2	<0.1	<0.1	<0.2	<0.1
	DH	T. tons.	AB	>10	<0.2	<0.1	>10	<0.2	<0.1	6.0	<0.2	<0.1
			SFM	<0.1	<0.2	<0.1	<0.1	<0.2	<0.1	<0.1	<0.2	<0.1
KM	IH	Dpt	AB	<0.1	<0.1	0.75	2.0	<0.1	1.7	<0.1	<0.1	1.4
			SFM	<0.1	<0.1	<0.75	<0.1	<0.1	<0.75	<0.1	<0.1	<0.75
MH	IH	Dpt	AB	<0.1	<0.3	<0.1	0.98	3.2	0.22	0.21	<0.3	0.16
			SFM	<0.3	<0.1	0.29	<0.3	<0.1	0.82	<0.3	<0.1	0.60
JN	IH	T. tons.	AB	0.17	1.95	0.10	—	—	—	0.15	1.8	0.17
			SFM	<0.1	<0.2	<0.1	—	—	—	<0.1	<0.2	<0.1
KA	DH	T. tons.	AB	4.9	<0.2	<0.1	>5.0	0.4	0.21	5.0	<0.2	0.20
			SFM	<0.1	<0.2	<0.1	<0.1	<0.2	<0.1	<0.1	<0.2	<0.1
AM	DH	T. tons.	AB	0.82	<0.1	0.99	4.2	<0.1	>10	1.5	<0.1	>10
			SFM	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

between *in vitro* responses that are enhanced in SFM and those that are not reflects the primary route of exposure.

A possible explanation is that this serum free media is enhancing the growth of a specific type of cell, which is in turn affecting proliferation in an allergen specific manner. Indeed, SFM was formulated for the culture of monocyte/macrophages and has also been successfully used to culture NK cells [25,34,35]. It is also possible that SFM is enhancing the growth of a specific cell type which is in turn secreting a proliferation enhancing or inhibitory cytokine. An inhibitory effect upon PBMC in PHA has been demonstrated with high levels of IL-10 or transforming growth factor- β [36–38]. It is uncertain whether the different effects upon proliferation seen with SFM are caused by the same mechanism. Preliminary experiments using cell depletion suggest that the effects of SFM on *Trichophyton* and tetanus toxoid proliferation are not dependent upon NK cells, monocytes, or B cells.

We conclude that SFM does not affect IH and DH T-cell responses differently and does not unmask T-cell responses in skin-test negative individuals to all allergens. Both IH and DH PBMC responses to *Trichophyton* were decreased at days 5 and 7 in SFM but not in AB media. However, SFM does appear to have distinct effects on different antigens. While the tetanus toxoid specific response was also decreased in SFM, there is a general increase in proliferation to dust mite antigens in both allergic and non-allergic subjects. The loss in proliferation to *Trichophyton* among DH subjects is accompanied by a decrease in the amount of IFN γ produced, however the detailed mechanism for the effect has not been resolved.

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