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Transplacental Priming of the Human Immune System to Environmental Allergens: Universal Skewing of Initial T Cell Responses Toward the Th2 Cytokine Profile[1]

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Transplacental Priming of the Human Immune System to Environmental Allergens: Universal Skewing of Initial T Cell Responses Toward the Th2 Cytokine Profile[1]

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The expression of Th2-skewed immunity against soluble protein Ags present in the normal environment is recognized as the primary cause of allergic inflammation in atopics. In contrast, nonallergic normal individuals display low level Th1-skewed immunity against the same Ags ("allergens"), which is perceived as conferring protection against Th2-dependent allergic sensitization. The type of T cell memory that develops against these Ags is currently believed to be the result of complex interactions between environmental and genetic susceptibility factors, which occur postnatally when the naive immune system directly confronts the outside environment. The results of the present study challenge this general concept. We demonstrate here for the first time that Th2-skewed responses to common environmental allergens, comprising IL-4, IL-5, IL-6, IL-9, and IL-13, are present in virtually all newborn infants and are dominated by high level production of IL-10. Moreover, these responses are demonstrable within 24 h of culture initiation, arguing against a significant contribution from covert in vitro T cell priming and/or differentiation. These findings imply that the key etiologic factor in atopic disease may not be the initial acquisition of allergen-specific Th2-skewed immunity per se, but instead may be the efficiency of immune deviation mechanisms, which in normal (nonatopic) individuals redirect these fetal immune responses toward the Th1 cytokine phenotype. ***The Journal of Immunology*****, 1998, 160: 4730–4737.**

The expression of Th2-skewed immunity to ubiquitous environmental allergens is the hallmark of the atopic phenotype and contrasts with the Th1-like pattern, which is stably expressed in normal adults throughout life (1, 2). These disparate patterns of T cell immunity are most clearly seen in responses to airborne allergens present in indoor environments, which are responsible for the bulk of allergic diseases within the community (3). The key question in relation to the etiology of atopic diseases is then: how are these alternate forms of Th memory against environmental allergens initially imprinted on the immune system, and in particular, what is the basis for selection of potentially pathogenic Th2-skewed memory in individuals genetically predisposed to atopy?

One of the central enigmas of the allergy literature is the concept of the "sensitization window" during infancy. This concept derives from a wide body of epidemiology literature (reviewed in Ref. 4) indicating that early postnatal exposure to high levels of allergen, exemplified by "birth during the pollen season," maximizes the risk for subsequent expression of allergic reactivity to that allergen in adult life. This suggests that allergen challenge of the immune system during infancy predisposes the child toward development of long term Th2-skewed allergen-specific immunologic memory. Additionally, the available evidence suggests that this risk is highest for children with atopic parents or siblings, i.e., for those with the atopic genotype (5).

Recent findings relating to the postnatal development of immune function have provided one possible explanation for this phenomenon. It has been demonstrated in a series of independent studies that PBMC from infants (6) and neonates (7–11) with positive atopic family histories have a diminished capacity for secretion of Th1 cytokines (in particular IFN-γ) relative to their family history-negative counterparts, and it has been suggested that delayed postnatal maturation of this important aspect of cellular immune function may be a key determinant of genetic predisposition to atopic disease (6, 12).

Consistent with this general postulate, a recent retrospective study (13) has demonstrated that expression of atopy in 12-yr-old children is most prevalent among those who had previously failed to develop Th1-dependent delayed tuberculin hypersensitivity in response to bacillus Calmette-Guérin (BCG) vaccination during infancy. Related to this issue, prospective seroepidemiologic studies on individual children (reviewed in Ref. 14) indicate that serum Ab responses against environmental allergens typically commence within a few months of birth, consistent with the initiation of primary immune responses as the naive immune system encounters these stimuli for the first time; a transient deficiency in Th1-related functions of the type reported (13) may accordingly increase the likelihood of selection for Th2-skewed memory during these early responses.

However, one further line of recent evidence suggests that there may be additional complexities in this process. Notably, low level lymphoproliferative responses to both inhalant and food allergens have been reported in cord blood (CB)[3] (8, 15, 16), suggesting that

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[3] Abbreviations used in this paper: CB, cord blood; HR, high risk; LR, low risk; CBMC, cord blood mononuclear cell; HDM, house dust mite allergen; DC, dendritic cell.

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initial priming of allergen-specific T cell responses may occur before birth. However, these claims are not universally accepted, as the origin of the putative allergen-reactive T cells in CB has not been formally established (for example, they may be of maternal origin), and there are no significant data on relevant T cell effector functions. The present study addresses these and related questions employing umbilical cord samples from 60 neonates, either with or without a family history of allergy, and for the first time provides formal proof of the fetal origin of the responding T cells as well as a comprehensive picture of their respective cytokine profiles.

Materials and Methods

Subjects

The study was approved by the Princess Margaret Hospital Ethics Committee (Perth, Western Australia). The CB donors were classified as genetically at high risk (HR) or low risk (LR) for allergy on the basis of a standardized questionnaire answered by the mothers, HR being defined as ≥1 first-degree relative with a positive allergy history (6, 17).

Preparation and stimulation of cord blood mononuclear cells (CBMC)

CB samples were collected after obtaining informed consent from the respective mothers. Collection was performed by inserting a 19-gauge needle into the placental vein, after careful swabbing with alcohol to remove all contaminating maternal blood. CBMC were cultured in triplicate for 7 days in microwells at 10^6 cells/ml in serum-free medium as detailed (18), either alone or in the presence of one of the following stimuli: unfractionated house dust mite allergen (HDM) extract (10 μg/ml), unfractionated rye grass pollen extract (WRE; 10 μg/ml), Fel d 1 allergen (30 μg/ml), OVA (100 μg/ml), β-lactoglobulin (10 μg/ml), or tetanus toxoid (0.5 Lf/ml); these concentrations were identified as optimal for in vitro T cell stimulation in preliminary experiments. DNA synthesis was measured as incorporation of [^{3}H]thymidine at the 7-day time point, after pulse labeling at 6 days. Data were expressed as δ dpm per culture, designating levels of incorporation above background (unstimulated) control cultures. Responses that were >2× background and >1000 dpm above background were considered positive (18).

To control for the potential stimulatory effects of LPS contamination in allergen extracts, a series of parallel studies was performed comparing allergen stimulation of CBMC in the presence and absence of polymyxin B at levels that blocked PBMC stimulation by 100 ng/ml LPS.

In a separate series of experiments, $CD4^+$ T cells were prepared from resting and allergen-stimulated CBMC employing $CD4^+$ Dynabeads (Dynal M-450; Dynal, Oslo, Norway) used at a bead:cell ratio of ≥4:1, and purified $CD4^+$ cells were subsequently released from the beads using Dynal Detach-a-bead reagent as per the manufacturer's instructions. The cellular content of the $CD4^-$ and $CD4^+$ fractions were monitored by flow cytometry, employing fluorochrome-conjugated anti-CD3, anti-CD4, and anti-HLA-DR. Where specified below, cell fractions were cultured with or without allergen, either alone or after recombination at various ratios.

Epitope analysis of CBMC response to OVA

A series of 28 sequential overlapping peptides spanning the OVA molecule, comprising 19 mers overlapping by 5, was synthesized. They were pooled in (sequential) sets of three and introduced into replicate microcultures at final individual concentrations of 10 μg/ml. [^{3}H]DNA synthesis was determined after 7 days, and individual responses were scored as positive/negative as described above.

Derivation and DNA typing of allergen-specific CBMC clones

T cell clones specific for the HDM of OVA allergen were derived from CB samples by methods based on those described previously (19). Briefly, allergen-induced T cell blasts were harvested from day 6 OVA- or HDM-stimulated cultures and cloned by limiting dilution in Terasaki microplates in the presence of X-irradiated heterologous splenocytes as feeder cells. Selected clones were expanded by polyclonal stimulation, rested, and retested for allergen specificity in a standard lymphoproliferation assay system using X-irradiated homologous T cell-depleted CBMC as APC, employing an unrelated Ag as control. The allergen-specific clones were cryopreserved until required for typing. For the latter procedure, genomic DNA was extracted into phenol-chloroform from parallel samples of 1) the clones, 2) PBMC taken from the mother of the original CB donor, and 3) PBMC from the CB donor at age 6 mo. One hundred nanograms of DNA was amplified by PCR using fluorescence-labeled primers for two loci. Data are reported herein for the *ACTBP2* locus (actin-binding protein microsatellite (20)). The PCR products were separated by electrophoresis in a 6% acrylamide sequencing gel, and the individual fragments were visualized by fluorescence and computer-assisted scanning.

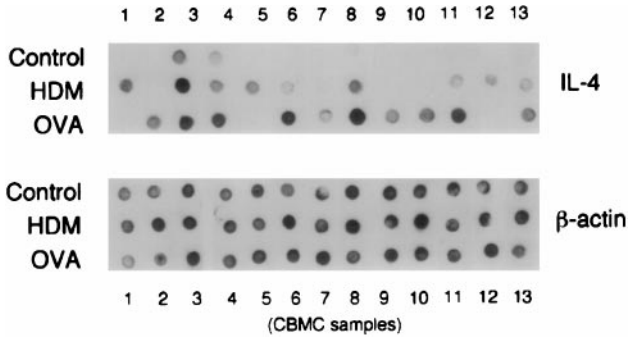

FIGURE 1. Dot blot analysis of IL-4 specific mRNA production in a representative series of CBMC samples stimulated with HDM or OVA allergen. RNA samples from control and stimulated cultures derived from 13 CBMC samples were reverse transcribed, amplified, blotted, and hybridized with a double-stranded probe specific for IL-4 as detailed in *Materials and Methods*.

Allergen-specific cytokine responses of CBMC

CBMC were cultured for 24 h in medium alone or in medium supplemented with optimal stimulating concentrations of HDM or OVA allergen. Supernatants were assayed for the presence of IL-6, IL-10, and IL-13 employing commercial ELISA kits (CLB Laboratories, Amsterdam, The Netherlands). For cytokines produced at lower levels (IL-4, IL-5, IL-9, and IFN-γ) together with β-actin, specific mRNA was measured in cell pellets by standardized semiquantitative RT-PCR as described (21).

Briefly, RNA was extracted from cell pellets using RNAzol B, and cDNA was transcribed using oligo(dT)15 and avian myeloblastosis virus reverse transcriptase in the presence of ribonuclease inhibitor. The PCR reactions, employing primers specific for IL-4, IL-5, IL-9, IFN-γ, and β-actin plus Tth *plus* (Biotech, Western Australia) polymerase, were performed in a programmable thermocycler (Perkin-Elmer, Norwalk, CT). Individual cycle analyses were performed with each primer set, and cycle numbers were chosen that were in the linear phase of the respective amplification reactions. Verification of the expected size of the PCR products was obtained by analysis in 1.5% agarose gels. Samples of the PCR products were manually dot-blotted onto nylon membranes and hybridized overnight with biotinylated double-stranded probes; binding was visualized by chemiluminescence using a commercial kit (ECL western blotting reagents, Amersham International Buckinghamshire, U.K.) (representative sample series shown in Fig. 1). Probe binding was quantitated by computer-assisted densitometric scanning. The intensity of each test sample was expressed as a ratio relative to that of its respective β-actin control.

Results

Lymphoproliferative responses to environmental allergens

CBMC responses to purified allergen preparations and tetanus toxoid Ag are shown in Figure 2. The majority (83%) of these 60 normal term fetuses show positive lymphoproliferative responses to one or more common allergens. The most frequent responses were to whole HDM extract (46%), β-lactoglobulin (44%), and OVA (42%). A proportion of samples also responded to Rye allergen (24%) and Fel d 1 allergen from cat (22%). A subset of the samples was tested with purified Der p 1 (the major allergen from HDM), and 73% displayed positive responses (not shown). Only 3% showed any response to tetanus toxoid. There was no difference in the frequency of positive responses in HR and LR samples at birth; however, the median, mean, and range of responses to all allergens were higher in the HR group, although these differences were not statistically significant using nonparametric tests.

Preliminary epitope mapping of putative fetal T cell responses to one selected allergen (OVA) was conducted on 22 of the CBMC

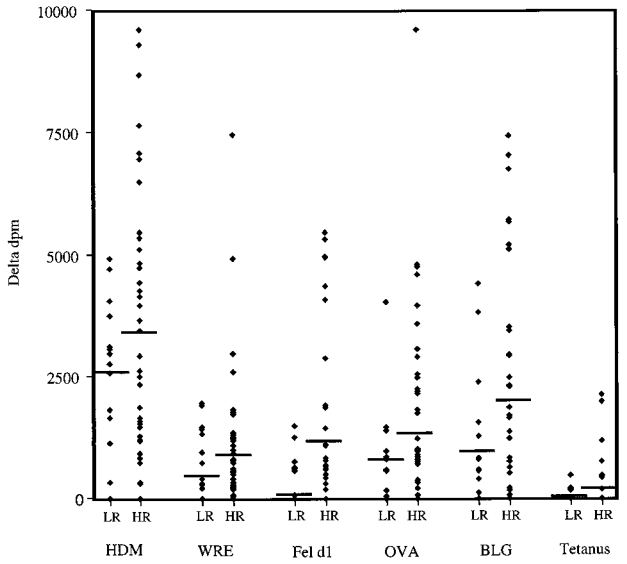

FIGURE 2. Lymphoproliferative responses to a range of allergens and Ags in CBMC. CBMC from donors genetically at high risk (HR) and low risk (LR) for atopy, on the basis of family history, were cultured for 7 days in microwells in the presence and absence of allergens, as detailed in *Materials and Methods*. DNA synthesis was measured as incorporation of [^{3}H]thymidine at 7 days and expressed as δ dpm per culture, designating levels of incorporation above background (unstimulated) control cultures. Each point shown represents the mean of triplicates from an individual CBMC sample.

samples to obtain further qualitative information relating to neonatal allergen recognition. These experiments utilized a set of 28 overlapping peptides spanning the OVA molecule. Due to limitations in the number of cells available per CBMC sample, it was not possible to test each CBMC against every one of the 28 OVA peptides individually, and hence for most of the experiments, the peptides were pooled sequentially in groups of three. Of the 22 CBMC tested, 14 responded to $\geq$2 of these peptide pools (Fig. 3), each of which contain multiple OVA epitopes. In a smaller follow-up series of 7 CBMC samples containing sufficient cells to test the full range of OVA peptides individually, an average of six peptides was recognized per CBMC (data not shown). This suggests that these fetal immune responses involve recognition of multiple regions within the OVA molecule and are thus likely to be directed against the native Ag, as opposed to a small number of epitopes in a cross-reacting Ag.

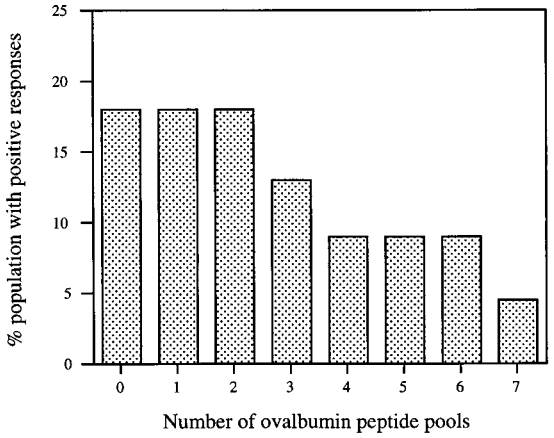

FIGURE 3. Crude epitope analysis of CBMC responses to OVA. Replicate samples from 22 CBMC were stimulated for 7 days with pooled OVA peptides as detailed in *Materials and Methods* before determination of [^{3}H]DNA synthesis. Positive responses were taken as those in which [^{3}H]thymidine incorporation levels in stimulated cultures were $\geq$twofold background and $>$1000 dpm above background. Responsiveness within the population tested is displayed as a frequency distribution histogram.

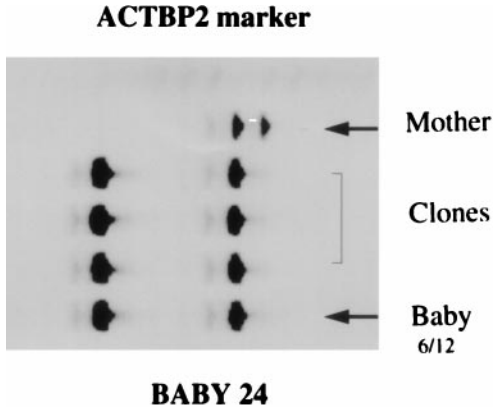

FIGURE 4. Microsatellite typing of DNA from putative fetal T cell clones. Extracts of DNA were analyzed by PCR amplification of polymorphic DNA markers actin-binding protein microsatellite ACTBP (illustrated), and also the marker D11S554 (not shown), as detailed in *Materials and Methods*. The PCR products were analyzed in a sequencing gel together with an allelic marker. The illustrated clones show polymorphic patterns that are identical to samples from the same donor at 6 mo of age and clearly distinct from respective maternal genotypes.

The experiments shown in Figure 4 address the hypothesis that CBMC preparations manifesting allergen-specific lymphoproliferative responses contain fetal T cells that exhibit the same Ag specificity. To test this hypothesis, T cell clones were derived from lymphoblasts harvested from bulk cultures of CBMC that were stimulated initially with OVA or HDM allergen and their respective specificities confirmed by subsequent restimulation with allergen in the presence of homologous APCs from cryopreserved stocks of T cell-depleted CBMC. DNA extracted from the putative fetal clones was compared with parallel samples from maternal cells and with PBMC from the CMBC donor collected at age 6 mo, using microsatellite genotyping at two polymorphic gene loci. Figure 4 demonstrates representative results from one of the two loci tested, clearly illustrating the fetal origin of the clones. Identical results were obtained via examination of the amplified products of the second locus. These findings were reproduced with an equivalent panel of clones from a second donor. These data provide the first direct proof that allergen-specific T cells in CBMC samples can be derived from the fetus and are not necessarily the result of contamination with maternal cells.

Cytokine production by allergen-stimulated CBMC

The principal focus of this study involved detailed analysis of cytokine production by allergen-stimulated CBMC in response to stimulation with the archetypal food allergen, OVA, and the archetypal inhalant allergen, HDM. Due to the expected low precursor frequency of allergen-reactive cells in these fetal responses, a minimum of 10^6 cells was used per test sample. Furthermore, in the light of the accumulating evidence that the selection of alternate pathways for Th cell differentiation during immune induction is markedly influenced by the cytokine milieu at the site of initial

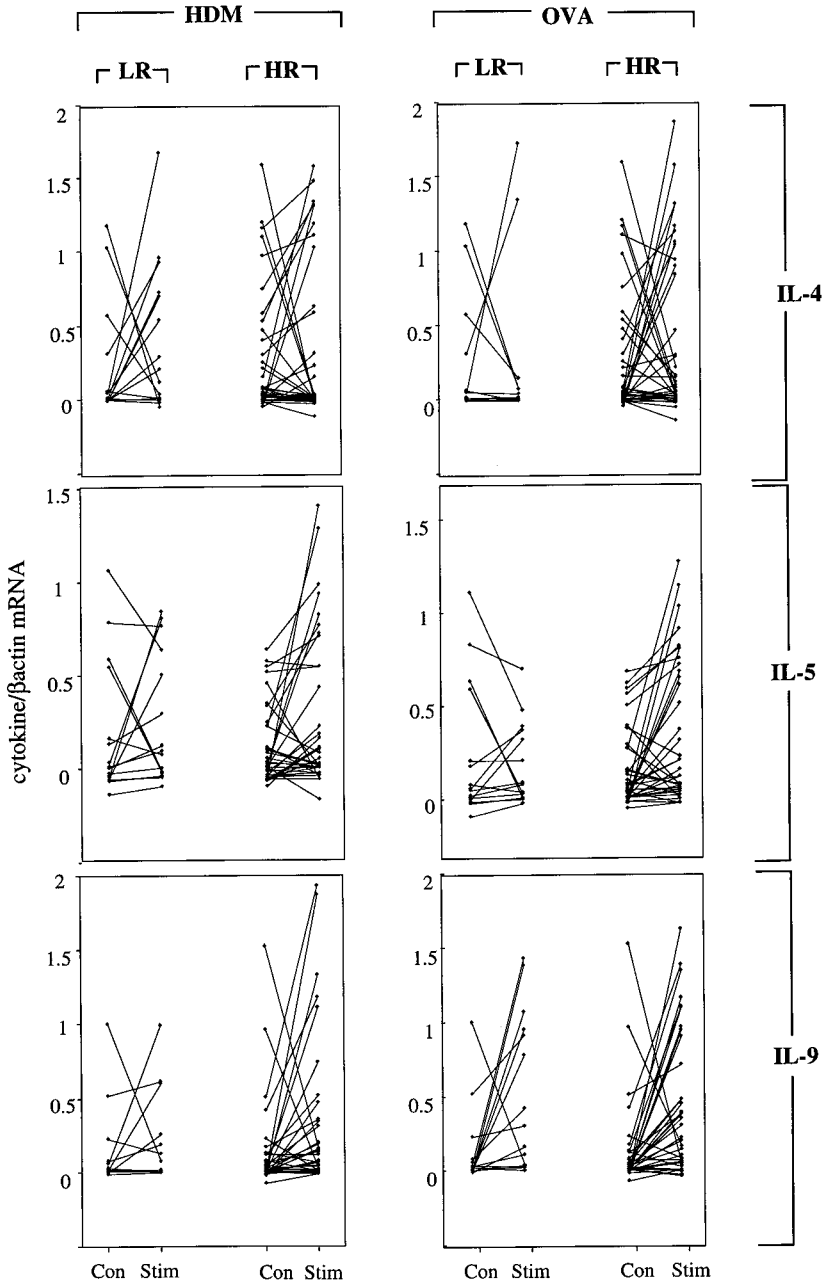

FIGURE 5. Allergen-specific cytokine mRNA production by CBMC. CBMC were cultured for 24 h in medium alone or in medium supplemented with optimal stimulating concentrations of HDM or OVA allergen. mRNA specific for β-actin, IL-4, IL-5, IL-9 (shown), and IFN-γ (not shown) were determined by standardized semiquantitative RT-PCR as detailed in *Materials and Methods*. Data are expressed as the ratio of cytokine-/β-actin-specific mRNA for individual samples from control (unstimulated) and parallel allergen-stimulated cultures (joined by solid lines).

T cell activation, we elected to focus upon the 24-h time point after in vitro T cell stimulation.

The levels of IL-4, IL-5, IL-9, and IFN-γ protein produced in these allergen-stimulated cultures were below the limits of detection by commercially available ELISA, necessitating reliance upon RT-PCR for semiquantification of specific mRNA. As shown in Figure 5, unequivocal up-regulation of mRNA production specific for IL-4, IL-5, and IL-9 was frequently observed in both LR and HR groups, employing 42 or 43 cycles for PCR. However, the use of at least 47 cycles was required for detection of allergen-induced IFN-γ signals, and IFN-γ-specific mRNA up-regulation was observed in only a small minority of allergen-stimulated cultures (not shown). This finding contrasts with our current experience in detecting equivalent IFN-γ responses in 5-year-old children and adults in whom 40 and 32 cycles, respectively, are sufficient for specific mRNA detection (Ref. 21 and experiments in progress).

ELISA screening of 24-h supernatants from allergen-stimulated cultures initially revealed that two cytokines, IL-10 and IL-13, were readily measurable at the protein level in samples from both LR and HR groups. Allergen-specific secretion of IL-13 protein was detected in cultures stimulated with either allergen, up to 10 pg/ml culture fluid (Fig. 6). However, these responses were overshadowed by parallel IL-10 production in the same allergen-stimulated cultures involving secretion of this cytokine at levels up to 3000 pg/ml culture fluid. This high level of production of IL-10 in allergen-stimulated bulk cultures was mirrored by results obtained with specific T cell clones (Table I). At the completion of this series of experiments, samples of supernatants from control and HDM-stimulated cultures remained available from 35 members of the HR group employed above. ELISA analysis of these supernatants indicated allergen-specific up-regulation of IL-6 secretion in 24 of the 35 samples tested, which was highly significant at the

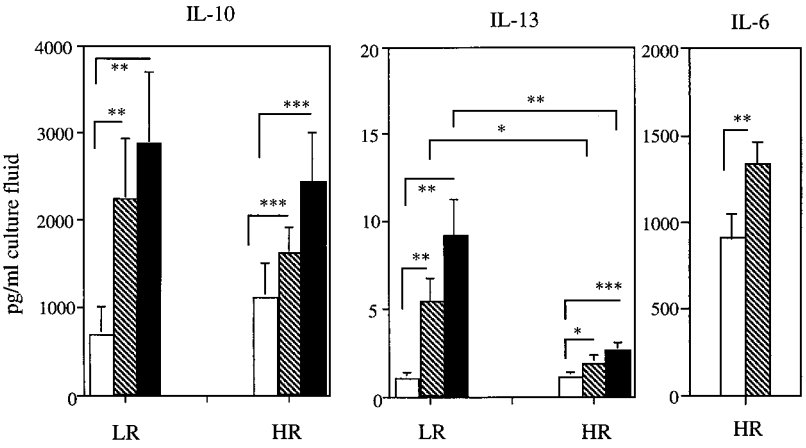

FIGURE 6. Allergen-specific production of IL-6, IL-10, and IL-13 protein. Supernatants from all cultures employed in the experiments of Figure 5 were initially analyzed for IL-10 and IL-13 protein using standard commercial ELISA kits. At the end of the study, samples remaining from 35 members of the HR group were analyzed for IL-6. Data shown represent group means ± SE. Open bars, background (unstimulated) cultures; hatched bars, cultures stimulated with HDM; black bars, cultures stimulated with OVA. Differences between group means were analyzed by *t* test: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

population level (Fig. 6). However, the clones produced no, or low levels of, IL-6, suggesting that the IL-6 production seen in the bulk cultures of CBMC may be from a non-T cell source. This possibility was further examined in the experiments described below.

Cellular source of allergen-specific cytokine production within CBMC in bulk culture

Three sets of experiments were performed to address this issue, focusing upon cytokines produced in sufficient amounts to be measurable as protein. First, to assess the possible effects upon cytokine production of contaminating LPS in allergen samples, we stimulated a series of CBMC with 100 ng/ml of LPS, or OVA or HDM at the levels used above, in the presence or absence of 1 μg/ml polymyxin B. Supernatants were collected at 24 h and assayed for IL-6 and IL-10; the polymyxin B inhibited LPS-induced cytokine production but did not affect stimulation by the allergens (data not shown).

Second, purified $CD4^+$ cells were separated from either resting CBMC or from CBMC cultures after a 20-h stimulation with HDM or OVA for further assessment of the relative roles of $CD4^+$ T cells vs other cell types in these cytokine responses. The $CD4^+$-enriched fractions used in the experiments below comprised 92 to 95% $CD3^+/CD4^+$ T cells with <2% $HLA\text{-}DR^+$ cells, and the $CD4^-$ fraction comprised 40 to 55% $HLA\text{-}DR^+$ cells with <3% $CD4^+$ cells. In the experiments shown in Figure 7, CBMC were precultured overnight with allergen before fractionation; the separated fractions were recultured in fresh medium for a further 24 h and supernatants assayed for cytokine. It is clear that IL-10 and IL-13 are exclusively produced by $CD4^+$ T cells in this system, whereas in contrast, >90% of IL-6 production is attributable to the $CD4^-$ cell fraction.

Table I. *Allergen-specific cytokine production by individual fetal clones*[a]

Cytokine	Clone No. 24/6	24/40	9/72
IL-10	31,000	9,400	≥ 50,000
IL-4	2,330	1,140	7,200
IL-13	9,200	7,400	12,500
IFNγ	1,360	6,090	2,580
IL-6	55	0	322

[a] Individual T cell clones from the series employed in Figure 2 were optimally stimulated with PHA/PMA, and 24-h culture supernatants were analyzed employing commercial ELISA reagents. Data shown represent cytokine concentrations in pg/ml.

The experiments shown in Figure 8 employed alternative methodology, focusing upon HDM-induced production of cytokines of potential monocyte origin, IL-6 and IL-10. In this case, separation and recombination of $CD4^+$ and $CD4^-$ fractions were performed before allergen stimulation. It is again clear that the $CD4^-$ fraction is capable of high levels of HDM-induced IL-6 production; however, it also appears that allergen-specific IL-6 production levels can be boosted markedly in the presence of T cells (cf IL-6 by 80×10^3 $CD4^-$ cells vs 80×10^3 $CD4^-$ T cells + 100×10^3 $CD4^+$ T cells), suggesting possible amplification of the $CD4^-$ IL-6 response via products from activated T cells. In contrast,

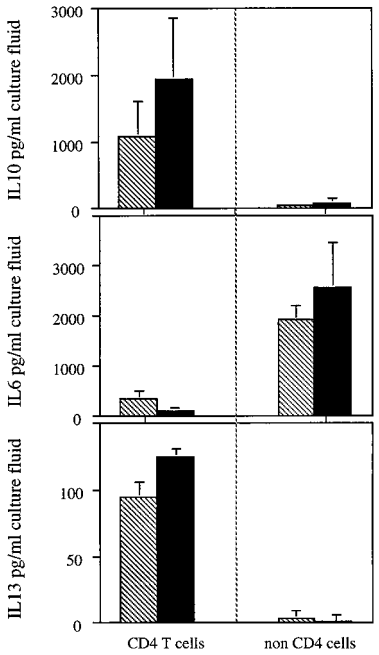

FIGURE 7. Allergen-specific cytokine production by T vs non-T fractions of CBMC: cell fractionation after allergen stimulation. CBMC were stimulated for 18 h with optimal levels of OVA or HDM in bulk culture in 1.0-ml, round-bottom tubes, harvested by aspiration, and fractionated into $CD4^+$ T/non-T fractions as described in *Materials and Methods*. The non-T fractions were returned to their original tubes, which contained adherent monocytes, and the $CD4^+$ T cells were placed in fresh tubes; secondary cultures were conducted for an additional 24-h (overnight) period in medium without further allergen supplementation, and supernatants were then assayed for cytokines. The data shown are mean ± SE from 5 samples. Hatched bars, HDM prestimulation; black bars, OVA prestimulation.

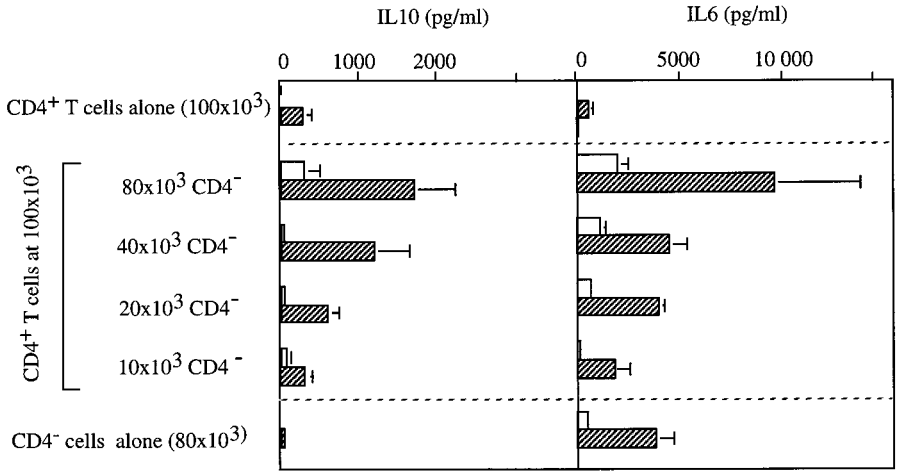

FIGURE 8. Allergen-specific cytokine production by T vs non-T fractions of CBMC: cell fractionation before allergen stimulation. Resting CBMC were fractionated into $CD4^+$ T and non-T fractions and recombined at the ratios shown before stimulation with HDM allergen. Supernatants (24 h) were collected for cytokine assays; data shown are mean ± SE from 5 samples.

$CD4^-$ cells do not make IL-10 in response to HDM, but their presence (presumably that of APCs) is required for the full T cell response.

The nature of the IL-6 response was not investigated further. However, given that we and others (22, 23) have shown that maternal allergen-specific IgG Abs are typically present in relatively high levels in CB, FcR-mediated stimulation of IL-6 secretion by cells such as monocytes would appear likely.

Discussion

Studies from several independent laboratories have demonstrated that CBMC from the majority of subjects respond positively to ubiquitous environmental allergens in lymphoproliferation assays (8, 15, 16). It is theoretically possible that the allergen-reactive cells in CBMC may be of maternal origin. If this were the case, it might be expected that the CBMC would also contain cells reactive to common vaccine Ags. However, the same CBMC samples generally fail to respond to the vaccine Ag tetanus toxoid (Ref. 16 and Fig. 2), against which the majority of adults in our population express active T cell immunity (18). This observation, taken together with the data presented above on DNA genotyping of allergen-specific T cell clones derived from stimulated CBMC samples, argues that the T cells that are responsive to these allergens in lymphoproliferation assays are of fetal as opposed to maternal origin. More importantly, this study provides new information on the underlying cytokine profiles in these fetal T cell responses. The latter experiments examined allergen-induced cytokine production at both the mRNA and (where feasible) protein level at 24 h poststimulation, to minimize the possibility of de novo priming of T cells in the cultures and/or their in vitro differentiation. Our results indicate that in contrast to the low level IFN-γ response characteristic of the majority of normal (nonatopic) adults (1, 2), the characteristic allergen-induced cytokine profile among neonates comprises IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, suggesting a generalized skewing of these allergen-specific immune responses toward the atopy-associated Th2 cytokine phenotype at this age.

It has not yet been established how these fetal responses are initiated, but one likely explanation is leakage of low levels of environmental allergens encountered during pregnancy (or peptides derived from them) across the placenta. Virtually all CB samples are rich in maternally derived IgG subclass Abs against common environmental allergens (22, 23), and the combination of the latter with an allergen may provide the initial signal for T cell priming. In this context, it is noteworthy that despite the presence of tetanus-specific IgG Abs in most CBs, tetanus-reactive T cells are rarely detected. Tetanus immunization during pregnancy is extremely uncommon, hence limiting the availability of relevant Ag to the fetus; significantly, when active tetanus immunization of pregnant women is performed, it results in tetanus-specific IgM production (indicative of the induction of primary immunity) in a high proportion of their offspring (24).

The finding that these fetal immune responses are dominated by Th2 cytokines is consistent with the recent literature indicating that the immunologic milieu at the fetomaternal interface is constitutively skewed away from Th1; this is believed to be an evolutionary adaptation aimed at protecting the fetoplacental unit against the toxic effects of cytokines such as IFN-γ (25, 26). It has been suggested that IL-10 production by mesenchymal cells within the placenta (23), in particular trophoblasts (27), plays an important role in this process, and its effects may be mediated via direct inhibition of IFN-γ production by T cells and/or via suppression of the Th1-selective functions (notably IL-12 production) of APCs (28, 29). These Th1-damping effects may be amplified via the production by the placenta of high levels of PGE_2, which selectively inhibits IFN-γ production (30–32), and further by local production of progesterone, which stimulates IL-4 production (33, 34).

Accordingly, exogenous Ags that leak across the placenta are likely to be presented to the fetal immune system within a milieu conducive to positive selection for Th2 immunity. Moreover, the ultra-low levels of Ag likely to cross the placenta are in the range that is also preferentially stimulatory to Th2 cells (35), and the initial skewing of fetal allergen-specific immune responses away from the Th1 cytokine phenotype may thus be the result of the tandem operation of these two factors. It is also of interest to note the high levels of allergen-specific IL-10 production by fetal T cells primed in this fashion (Fig. 6 and Table I), which may serve to reinforce this Th2-polarization during subsequent restimulation driven by early postnatal encounters with these allergens. In this context, high levels of microenvironmental IL-10 during T cell activation have also been demonstrated to promote anergy development, particularly in the presence of potent T cell stimuli such as alloantigen (36), and a similar mechanism may contribute to the postnatal regulation of T cells to responses driven by direct stimulation with some environmental allergens. One example of the latter may be responses to Ags present at high concentrations in the diet, which typically peak during early childhood and wane thereafter, suggesting the operation of underlying negative control mechanism(s) (14, 16).

These findings have important implications for the etiology of atopic diseases. As noted above, early postnatal allergen exposure

is a risk factor for development of long term, primary allergic sensitization, and the present study suggests that the basis for this risk may be the presence in the newborn of small populations of allergen-specific T cells that are primed during intrauterine life. This early Th2 priming, as described in recent murine models of neonatal tolerance (37, 38), is capable of deviating subsequent immune responses toward the selection of potentially pathogenic Th2-polarized memory.

The available epidemiologic evidence also indicates that the risk for development of Th2-polarized memory against environmental allergens is highest in subjects of the atopic genotype, i.e., those with a positive atopic family history (5, 39). It is feasible that the latter may be a reflection of the degree of polarization of initial allergen-specific Th responses during fetal life; this possibility provided the impetus for the LR:HR comparisons in the present study. While our observations in Figure 5 show a general tendency toward higher allergen-specific IL-5 and IL-9 responses within the HR population, the differences are not consistent and may reflect the small size of the LR group. It is pertinent to note in this context that several reports have demonstrated that generalized capacity for IFN-γ production, as measured in PHA stimulated cultures, is reduced in CBMC samples from HR donors relative to those from LR subjects (7–11). This important issue clearly requires a more detailed investigation to elucidate the differences between individuals that relate to genetic background.

Additionally, this study highlights the potential importance of developmental factors associated with the postnatal maturation of overall immune competence in relation to the ultimate expression of the atopic phenotype. Thus, our results indicate that virtually all infants are born with their immune systems primed for the development of potentially pathogenic immunologic memory against environmental allergens, and yet resulting Th2-dependent allergic disease eventually manifests in only a minority of (atopic) subjects, as the majority eventually develops low level Th1-polarized memory to the same allergens (1, 2).

This implies that allergen responder phenotype is determined, to a substantial degree, by immune deviation involving allergen-driven T cell selection after birth, when the weakly primed neonatal immune system is confronted with (relatively) high levels of incoming allergens from the outside environment (14). This T cell selection process is dependent upon the functions of relevant APC. In the case of inhalant allergens, which are responsible for the bulk of atopic disease, the principal APC population involved are intraepithelial dendritic cells (DC), which occur as a dense network throughout the airway mucosa (40–42). It is noteworthy that this cellular network is poorly developed at birth and matures relatively slowly postnatally; during the preweaning period, the airway DC exhibit low MHC class II expression, poor APC function, and are refractory to inflammatory stimuli relative to the same cells in adults (4, 43, 44).

These latter findings are mirrored by recent reports on the role of DC in the neonatal tolerance phenomenon in mice, which have demonstrated that DC populations from central lymphoid organs in newborn animals are deficient in the capacity to prime naive T cells for Th1-dependent memory generation (37), resulting in a generalized skewing of immune responses during infancy toward the Th2-cytokine profile (37, 38, 45, 46). The capacity of human peripheral tissue DC to redirect neonatally primed T cell responses against environmental (particularly inhalant) allergens toward the nonatopic Th1 profile may, by analogy, be intrinsically low at birth and may additionally be further compromised by the high levels of IL-10 produced by these allergen-primed T cells during postnatal stimulation.

The precise role of APC in the postnatal maturation of Th1-associated functions in humans has not yet been established. However, the generalized poor in vitro performance of peripheral blood APC from human infants has been recognized for some years (47). Moreover, the results of in vitro cell mixing experiments suggest that the low capacity of infant peripheral blood T cells to produce IFN-γ in response to polyclonal stimulation is attributable, to a significant extent, to a transient developmental defect in the costimulatory function(s) of their endogenous accessory cells (48, 49), and consequently, it appears that the parallels with the murine system may be strong.

It is reasonable to speculate that the longer APC populations in human infants continue to express the neonatal functional phenotype during postnatal life, the greater will be the potential for consolidating allergen-specific Th2-polarized memory. The nature of the genetic and environmental factors that normally regulate the kinetics of postnatal development of DC remains to be elucidated, but a deeper understanding of the underlying mechanisms may provide novel opportunities for the primary prevention of these diseases, for example via hastening postnatal maturation of their Th1-stimulating functions. In this context, attention has recently been focused on the apparent inverse relationship between infections during early life and the subsequent expression of Th2-dependent allergy (5, 14, 50–52), and we speculate that these effects may be mediated in part via infection-driven stimulation of the Th1-associated immune functions of DC and other APC, such as IL-12 production.

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