

Cells and mediators involved in immunoglobulin synthesis by human circulating mononuclear cells

IV. B cells synthesize but do not secrete immunoglobulins because of a defect in the non-T non-B (null) cells

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Null cells (non-T and non-B lymphocytes) have previously been demonstrated to be obligatory participants for immunoglobulin synthesis and secretion by normal B cells in culture. Normal null cells have been demonstrated to secrete a factor, human immunoglobulin synthesis/secretion-facilitating factor (HISFF), which can replace the null cells in the culture. In this investigation, the B cells of an 8-month-old male infant and a 46-year-old male adult who presented with a humoral (antibody) immunodeficiency syndrome synthesized immunoglobulin but did not secrete immunoglobulin after culture with pokeweed mitogen and autologous T cells, monocytes, and null cells. In contrast, the patients' B cells synthesized and secreted immunoglobulin after the addition of allogeneic normal null cells or HISFF to the cultures. The same results were obtained with the cells of the infant and the adult patient tested at monthly intervals for 4 months. The results demonstrate that the patients' T cells, B cells, and monocytes functioned normally and that only the patients' null cells were defective. These findings provide an explanation for the absence of immunoglobulin in the circulation of "non(immunoglobulin)secretors," although they possess normal numbers of circulating immunoglobulin-synthesizing B cells. The defect is in the null cell and not in the B cell and consists of the inability of the null cell to secrete HISFF that facilitates the synthesis and secretion of immunoglobulin by the B cell. (J ALLERGY CLIN IMMUNOL 1992;90:301-12.)

Key words: Immunodeficiency, null cells, immunoglobulin synthesis

The null cells, also referred to as (1) non-T and non-B cells, (2) L cells, and (3) third-population cells (see DISCUSSION), are obligatory participants, in addition to T_H helper cells, monocytes, and PWM, in the synthesis and secretion of immunoglobulins by B cells in culture.^{1,2} Null cells secrete a factor, HISFF,

which can replace the null cells in culture.³ HISFF is not secreted by T cells, B cells, or monocytes, whether they are cultured individually or in combination, and HISFF cannot be replaced by the known interleukins.³ HISFF is not HLA restricted in its activity.³

In this investigation, we demonstrated that the B cells of an 8-month-old male infant and of a 46-year-old male adult who presented with a humoral immunodeficiency syndrome synthesized immunoglobulin but failed to secrete immunoglobulin in culture because of a defect in the null cells. The infant and the adult patient were each retested at monthly intervals for 4 months, and the identical results were obtained.

MATERIAL AND METHODS

Patients

Patient No. 1. An 8-month-old white male infant was admitted with a 2-week history of tachypnea, dyspnea, and dry cough, but no fever. There was no previous history of

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serious bacterial or viral illnesses. Physical examination, on admission, revealed a well-nourished male infant of normal length in moderate respiratory distress. Chest x-ray film revealed consolidation of the right lung and numerous infiltrates involving the left lung. He had a normal thymus. The white blood cell count was markedly elevated ($39.8 \times 10^9/L$). Although the percentage of lymphocytes (27%) was in the normal range, the absolute number of lymphocytes ($10.7 \times 10^9/L$) was markedly elevated. Approximately 60% of the lymphocytes were B cells (CD19 and CD20 positive) and 30% were T cells (CD2 and CD3 positive), indicating a B cell lymphocytosis but normal numbers of T cells. About 10% of the lymphocytes were non-T and non-B cells or null cells (CD16 and CD56 positive). Circulating IgG and IgA were barely detectable (0.07 gm/L and <0.03 gm/L, respectively), whereas circulating IgM (0.13 gm/L) was markedly below the normal range for infants in the patient's age group. Similar results were obtained on four different occasions during a period of 4 weeks. Isoagglutinins were not assayed because the patient was AB positive.

The respiratory infection cleared up after the administration of septrin, 0.25 ml by mouth, every 6 hours for 10 days. Because of the low levels of circulating immunoglobulins, gammaglobulin was administered intravenously and repeated at weekly intervals for 4 weeks. At the time of discharge, the concentration of circulating IgG was 5.2 gm/L.

Patient No. 2. A 46-year-old white man who had been in good health until 3 to 4 years previously, when he began to experience repeat bouts of coughing, often accompanied by fever. The infections cleared after treatment with broad-spectrum antibiotics. Immunoglobulin determinations on two occasions 3 months apart revealed that all the major immunoglobulin classes were markedly below their normal ranges. IgG levels were 0.84 and 0.93 gm/L, IgA levels were <0.07 gm/L, and IgM levels were 0.10 and 0.12 gm/L (normal values for IgG are 6 to 20 gm/L; IgA, 2.0 to 4.5 gm/L; and IgM, 0.75 to 1.5 gm/L).

Isolation or elimination of cells

The methods for (1) the isolation of the MNCs from the blood, (2) the isolation of the T cells by rosetting the MNCs with SRBC, (3) the isolation of the T_H helper cells by rosetting the T cells after incubation at 37° C for 24 hours with EAM indicator cells (see below), (4) the isolation of the null cells and the B cells by rosetting the non-T cells with EAG and EAC indicator cells (see below), respectively, and (5) the elimination of monocytes from the MNCs were previously described in detail.^{2,3}

Preparation of EAG indicator cells for the isolation of the null cells

The EAG indicator cells are the IgG antibody-sensitized ORBCs and were prepared as described by Froland et al.⁴

Preparation of EAM indicator cells for the isolation of the T_H helper cells

The EAM indicator cells are the IgM antibody-sensitized ORBCs and were prepared as described by Moretta et al.⁵

Abbreviations used

PWM:	Pokeweed mitogen
IL:	Interleukin
SRBC:	Sheep red blood cells
ORBC:	Ox red blood cells
EAG:	Ox erythrocytes coated with IgG antibodies
EAM:	Ox erythrocytes coated with IgM antibodies
EAC:	Ox erythrocytes coated with subthreshold IgM antibodies and complement
FCS:	Fetal calf serum
T_H :	T cell with receptor for FcM
IF:	Immunofluorescence
HISFF:	Human immunoglobulin synthesis/secretion-facilitating factor
NHISFF:	HISFF secreted by normal null cells
PHISFF:	HISFF secreted by patient's null cells
MNC:	Mononuclear cells
PMNC:	Patient mononuclear cell
Mab:	Monoclonal antibodies
NK:	Natural killer
PBS:	Phosphate-buffered saline
PWM:	Pokeweed mitogen

Preparation of EAC indicator cells for the isolation of the B cells

The EAC indicator cells are the complement (C)-bound antibody(IgM)-sensitized ORBCs, and were prepared as described by Ross et al.⁶

The culture conditions for optimal immunoglobulin synthesis and secretion

The culture conditions for optimal IgG synthesis and secretion have been described in detail previously.^{2,3} In brief, the T_H cells (2×10^5 cells) were incubated with an equal number of non-T cells (2×10^5 cells) in 1 ml of culture medium ([RPMI 1640] fortified with fetal calf serum, final concentration, 20%, penicillin [100 U/ml], and streptomycin [100 µg/ml]) in sterile polystyrene tubes (Falcon 2004, Becton Dickinson Canada Ltd., Mississauga, Ontario). PWM (0.05 ml) was added in a concentration that promotes maximum immunoglobulin synthesis and secretion by normal B cells in culture. The cultures were maintained in a stationary phase at 37° C in 5% CO₂ in air for 7 days.

Unless it is stated otherwise, monocytes were present in the cultures in a concentration of 8% to 15%. All cultures were set up in duplicate, or in triplicate if there were sufficient cells.

The T_H cells were used as T helper cells rather than the CD4-positive T cells. The CD4-positive T cells, which constitute 60% to 70% of the T cells, include the T_H cells (30% to 40% of the CD4-positive cells),⁷ the suppressor-inducer (CD45R) cells,⁸⁻¹⁰ the helper-inducer (CDw29) cells,^{11,12} the Tar (autologous rosetting T) cells,^{13,14} the T-sensitized cells, the CD4 cells that rosette with EAG,¹⁵ and other as yet

unidentified cells. It can be consistently demonstrated that the CD4-positive cells depleted of T_M cells lose all helper-cell activity and, conversely, that all the helper cell activity is exhibited by the T_M cells (unpublished results). The T_M cells are therefore a more homogenous population of helper T cells than are the unfractionated CD4-positive T cells.

One percent to 3% of the T_M cell population reacted with the B cell-specific CD19 and CD20 MAbs and 3% to 5% reacted with the monocyte-specific CD14 MAbs (unpublished results). The T_M cells are therefore only minimally contaminated with B cells and monocytes.

The detection of immunoglobulin synthesis and immunoglobulin secreted by the cultured B cells

The detection of immunoglobulin synthesis (cytoplasmic immunoglobulin) by the cultured B cells by IF and immunoglobulin secreted by the cultured B cells by ELISA have been described in detail previously.^{2,3} Briefly, the cultured cells were centrifuged at 300 g, and the supernatants were carefully collected and transferred to other tubes for the determination of IgM and IgG by ELISA. The cells were washed twice with RPMI 1640, resuspended in 100 μ l of a 25% solution of human albumin, and cytocentrifuged on microscope slides in a Shandon (Shandon, Inc., Pittsburgh, Pa.) cytocentrifuge. The cells were fixed on the slides by incubation in a cold 5% solution of acetic acid in ethanol for 10 minutes at 4° C. The slides were rinsed in PBS at room temperature and then incubated with fluorescein isothiocyanate-conjugated goat antibodies to total human immunoglobulin (IgG, IgM, and IgA) (Organon Teknika, West Chester, Pa.) for 30 minutes at room temperature. The slides were then rinsed in PBS and examined by fluorescence microscopy. The percentages of IF-positive cells in the duplicates or triplicates performed invariably differed by <10%.

Immunoglobulin secreted into the culture medium by the B cells was determined by ELISA. Inert disks coupled with goat antibodies to human IgM (or IgG) (Dimension Laboratory, Inc., Toronto, Ontario) were incubated with aliquots of the cell-free culture supernatants (step 1). After a 60-minute incubation period, the disks were washed five times with PBS and incubated with goat antibodies to human IgM (or IgG) complexed with alkaline phosphatase (step 2). After another 60-minute incubation period, the disks were washed five times with PBS, transferred to tubes containing the chromogen, p-nitrophenyl phosphate, and incubated for an additional 60 minutes (step 3). The reaction was terminated by the addition of 3 mol/L of NaOH. The optical density was read in a colorimeter at 405 nm and converted to nanograms of immunoglobulin per milliliter.

In several of the tables, only the results for IgM secreted by the cultured B cells are presented since the amounts of IgM secreted by the B cells were, invariably, consistently observed to be three to five times the amounts of IgG secreted, and IgG was never secreted in the absence of IgM.

The variation in the amounts of immunoglobulin secreted between the duplicate or triplicate cultures rarely exceeded 10%.

T_M and null cell-culture supernatants

T_M cells (10^6 cells) or null cells (10^6 cells) were cultured with PWM for 7 days in 1 ml of culture medium in sterile polystyrene tubes (Falcon, No. 2004) at 37° C in 5% CO₂ in air. Monocytes were present in a concentration of 4% to 10%. At the termination of culture, the tubes were centrifuged at 400 g for 10 minutes. The cell-free supernatants were filtered through 0.22 micropore-sized Nalgene filters (Nalgene Labware, Rochester, N.Y.) and stored at -20° C if they were not used immediately.

Preparation of NHISFF and PHISFF, respectively

Null cells (5×10^5) were cultured for 7 days at 37° C in 5% CO₂ in air under the following conditions: (1) with T_M cells (5×10^5) and PWM in 1 ml of culture medium or (2) in 1 ml of the T_M cell-culture supernatant containing PWM. At the termination of culture, the tubes were centrifuged at 400 g for 10 minutes. The cell-free supernatants were filtered through 0.22 micropore-sized Nalgene filters and stored at -20° C if they were not used immediately.

Detection of HISFF

HISFF was detected by its ability to promote immunoglobulin synthesis and secretion by B cells cultured for 7 days with T_M helper cells, monocytes, PWM, and the HISFF to be assayed.³ In the absence of HISFF, immunoglobulin is neither synthesized nor secreted by the cultured B cells.³

Identification of rosetted cells with MAbs

The T, B, and null cells isolated by differential rosetting were investigated for cell-surface markers with the following MAbs (Becton Dickinson Canada Ltd.): CD2, CD3, and CD5 MAbs (specific for T cells), CD19 and CD20 MAbs (specific for B cells), CD14 MAbs (specific for monocytes), and CD16 and CD56 MAbs (specific for NK cells). The CD16 and CD56 were combined. The antibodies were conjugated with fluorescein or phycoerythrin, and the labeled positive cells were detected and quantified with a Becton Dickinson FACScan flow cytometer.

Each experiment was performed in duplicate a minimum of three times. The figures presented in the tables are the actual results obtained in the particular experiment(s) and are representative of all the experiments performed. The results presented in Fig. 1 are based on the results obtained with the cells of >100 normal adult volunteers.

RESULTS

The relationship between immunoglobulin synthesis and immunoglobulin secretion by normal B cells in culture

Normal B cells were cultured with autologous T_M cells, null cells, monocytes, and PWM under culture conditions optimal for immunoglobulin synthesis and secretion (see MATERIAL AND METHODS). As illustrated in Fig. 1, a small percentage of the freshly isolated circulating cells (<5%) are IF positive (immunoglobulin-synthesizing cells) but they disappear

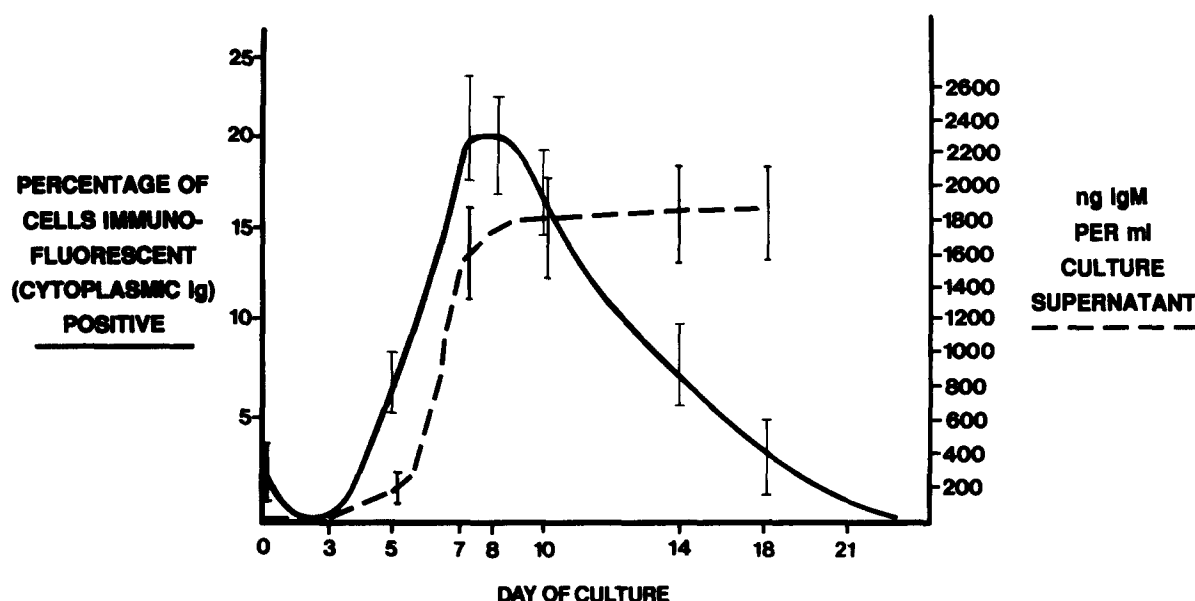


FIG. 1. Synthesis and secretion of immunoglobulins by normal B cells in culture. Means and ranges are based on results obtained with cells from more than 100 normal adult volunteers.

by day 2 of culture. IF positive cells can be once more detected by day 3 of culture. The percentage of IF-positive cells then increases rapidly to reach a maximum by day 7 or 8 of culture, indicating intense immunoglobulin synthesis by the cultured B cells. The secretion of immunoglobulin by the cultured B cells parallels the immunoglobulin-synthesis curve defined by IF. Insignificant amounts of immunoglobulin are secreted before day 5 of culture, but immunoglobulin secretion then increases rapidly to reach a peak between days 7 and 8 of culture.

The relationship between the percent of IF-positive cells and the amount of immunoglobulin secreted varies only minimally from one normal individual to another. We have not yet encountered a normal individual with IF-positive B cells on day 7 of culture whose B cells did not secrete immunoglobulin into the culture medium nor whose B cells secreted immunoglobulin in the absence of IF-positive B cells.

Infant patient's B cells require allogeneic normal null cells or NHISFF to secrete immunoglobulin

The infant patient's B cells, cultured with PWM, autologous T cells, null cells, and monocytes (PMNCs), synthesized, but did not secrete, immunoglobulin, irrespective whether (1) normal T_M cells, or (2) normal T_M cell-culture supernatant, or (3) normal T_M cells and normal B cells (both of which were contaminated with normal monocytes), were added to

cultures of patient's cells at the beginning of the culture period (Table I). In contrast, the patient's B cells (PMNCs) synthesized and secreted immunoglobulin after the addition, at the beginning of the culture period, of either (1) normal null cells and normal T_M cells or (2) normal null cells and normal T_M cell-culture supernatant, or (3) NHISFF (Table I). In contrast, the patient's B cells (PMNCs) cultured in PHISFF synthesized, but did not secrete, immunoglobulin (Table I).

The results obtained with the cells of the infant patient on a second occasion 1 month later (aged 9 months) are presented graphically in Fig. 2. Results are very similar to those presented in Table I. However, this presentation of these data permits a clearer appreciation of the facilitating or stimulating effect that the added normal null cells or NHISFF have on the patient's B cells to secrete the immunoglobulin that they can normally synthesize but cannot secrete in vitro and in vivo. It must be noted that the normal B cells (normal MNCs) were obtained from a normal adult. The amounts of immunoglobulin secreted by the B cells of normal adults are four to eight times greater than the amounts of immunoglobulin secreted by the B cells of infants younger than 1 year of age. In point of fact, the amount of immunoglobulin secreted by the patient's B cells in the presence of NHISFF (PMNC plus NHISFF, Fig. 2) is representative of the amounts of immunoglobulin secreted by normal infants' B cells in culture.

TABLE I. The failure of immunoglobulin secretion by the infant patient's B cells is caused by defective null cells (data are of a representative experiment of a total of four experiments performed)

Cultures*	% Ig-synthesizing B cells†	Ig synthesis and secretion by cultured cells	
		ng Ig/ml‡	
		IgM	IgG
Normal (N) adult control subject NMNC	26	1534	320
Patient (P)			
PMNC§	24	<56	<11
PMNC§ + NT _M cell-culture supernatant	22	<56	<11
PMNC + NT _M	21	<56	<11
PMNC + NB + NT _M	26	<56	<11
PMNC + Nnull + NT _M	21	497	97
PMNC + Nnull + NT _M cell-culture supernatant¶	20	462	80
PMNC§ + NHISFF	23	452	85
PMNC§ + PHISFF	24	<56	<11
Normal (N) age-matched control subjects (age)			
NMNC§ (5 months)	15	215	ND
NMNC§ (6 months)	14	170	ND
NMNC§ (8 months)	19	310	ND
NMNC§ (11 months)	17	267	ND
NMNC§ (12 months)	23	323	ND
NMNC§ (12 months)	24	340	ND
Mean	18	271	
SD	4.2	67	

Ig, Immunoglobulin; NB, normal B cells; ND, not done.

*The cells were cultured for 7 days in culture medium fortified with PWM. The concentration of monocytes was 8% to 15%.

†The percent immunoglobulin-synthesizing (cytoplasmic immunoglobulin positive) cells determined by IF.

‡Nanograms per milliliter of cell-free culture-supernatant determined by ELISA.

§Cells (4×10^5) per milliliter of culture medium.¶The T_M cell-culture supernatant did not contain detectable amounts of IgM or IgG.

Almost identical results were obtained with the cells of the infant patient assayed on two additional occasions, at age 10 and 11 months.

The B cells of normal age-matched infants synthesize and secrete immunoglobulin

The B cells obtained from six consecutive normal infants, 5 to 12 months of age, cultured with PWM and autologous T cells, null cells, and monocytes, synthesized and secreted significant amounts of immunoglobulin (Table I).

Adult patient's B cells require allogeneic null cells or NHISFF to secrete immunoglobulin

The adult patient's B cells synthesized immunoglobulin but secreted insignificant amounts of immunoglobulin when B cells (PNon-T) were cultured with autologous T cells, null cells, and monocytes (Table II, PMNCs, PT plus Pnon-T). The patient's B

cells also failed to secrete immunoglobulin in cultures enriched with normal T cells and monocytes (NT plus PNon-T). The failure of the patient's B cells to secrete immunoglobulin cannot be attributed to suppressor cells since the patient's MNCs cultured with normal MNCs did not suppress the normal synthesis and secretion of immunoglobulin by the normal B cells (Table II). Furthermore, the patient's T cells cultured with normal non-T cells (PT + NNon-T, Table II) did not inhibit either the synthesis of immunoglobulin or the secretion of normal amounts of immunoglobulin by the normal B cells.

However, the patient's B cells secreted copious amounts of immunoglobulin when NHISFF was incorporated into the culture (Table II). Normal B cells (Nnon-T cells) synthesized and secreted immunoglobulin normally after culture with the patient's T cells, indicating that the patient's T cells were not defective. These results therefore indicate that failure

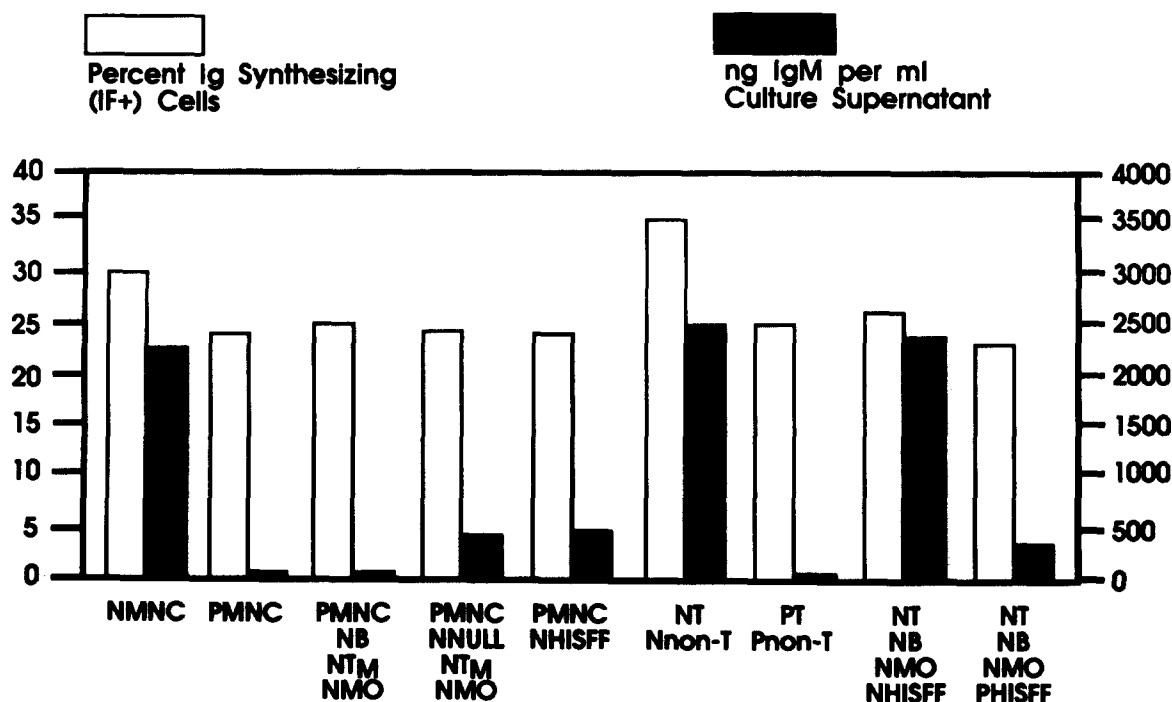


FIG. 2. Infant patient's B cells synthesize and secrete immunoglobulins in the presence of normal null cells or normal HISFF; *N*, normal; *P*, patient; *MO*, monocytes. Large amounts of immunoglobulin secreted by normal B cells is attributed to the fact that they were obtained from a normal adult.

of immunoglobulin secretion by the patient's B cells in culture with autologous T cells, null cells, and monocytes may be attributed to a null cell defect.

The T_M cell culture supernatant can replace T cells for the synthesis and secretion of HISFF by cultured normal null cells

Normal B cells were cultured with normal monocytes, PWM, and (1) normal null cells, (2) normal T_M cells, (3) normal unfractionated T cells, (4) the normal T_M cell-culture supernatant, or (5) the normal null cell-culture supernatant. These B cells did not synthesize or secrete immunoglobulin (Table III). However, the B cells cultured with normal monocytes, PWM, and (1) normal null cells and T_M cells or (2) normal null cells and the T_M cell-culture supernatant, synthesized and secreted immunoglobulin normally (Table III), indicating that HISFF had been secreted into the culture medium. In contrast, the B cells cultured with T_M cells, monocytes, and the null cell-culture supernatant did not synthesize or secrete immunoglobulin (Table III). It should be emphasized that neither the null cell-culture supernatant nor the T_M cell-culture supernatant contains HISFF, since B cells cultured in these supernatants did not synthesize or

secrete immunoglobulin (Table III). It must be noted that no IgM or IgG was detected in the T_M cell-culture supernatant or in the null cell-culture supernatant. These results clearly demonstrate therefore that HISFF is secreted by the null cells cultured in the T_M cell-culture supernatant in response to a signal secreted by the T_M cell thereby facilitating immunoglobulin synthesis and secretion by the B cells.

The phenotypes of the isolated normal T, B, and null cells

Approximately 85% to 92% of the T cells isolated by rosetting with SRBCs were CD2, CD3, and CD5 positive; <4% of the cells were CD19/CD20 positive, and 15% to 25% were CD16/CD56 positive (Table IV).

Most B cells isolated by rosetting with EAC were CD19/CD20 positive. Six percent to 12% were CD16/CD56 positive, and <2% of the cells were CD2/CD3 positive (Table IV).

Approximately 4% to 10% of the null cells isolated by rosetting with EAG were CD19/CD20 positive and <1% were CD2/CD3 positive, indicating contamination with only a small number of B cells. Most null cells were CD16/CD56 positive (Table IV). These

TABLE II. The limited synthesis and secretion of immunoglobulins by adult patient's B cells is caused by defective null cells (data are of a representative experiment of a total of four experiments performed)

Cultures*	Ig synthesis and secretion by the cultured cells	
	% Ig-synthesizing B cell†	ng IgM/ml‡
NMNC§	24	1838
NT + Nnon-T	27	2063
PMNC§	9	177
PMNC§ + NMNC§	30	2480
PT + Pnon-T	11	194
PT + Pnon-T + NHISFF	20	1492
NT + Pnon-T	10	156
PT + Nnon-T	18	1262

Ig, Immunoglobulin; P, patient; N, normal control subject.

*The cells were cultured with PWM for 7 days in culture medium.

†The percent immunoglobulin-synthesizing (cytoplasmic immunoglobulin positive) cells determined by IF.

‡Nanograms of IgM per milliliter of cell-free culture supernatant determined by ELISA.

§MNCs (4×10^5) per milliliter of culture medium.

||Cells (2×10^5) per milliliter of culture medium.

TABLE III. The T_M cell-culture supernatant can replace T_M cells in the synthesis of HISFF by normal null cells (data are of a representative experiment of a total of three experiments performed)

Cultures*	% Ig-synthesizing B cell†	Ig synthesis and secretion by the cultured cells	
		ng Ig/ml‡	
		IgM	IgG
NMNC§	20	2147	394
NB + NT _M + Nnull§	22	2380	428
NB + NT + Nnull§	22	2262	412
NB + NT	1	<56	<11
NB + NT _M	0	<56	<11
NB + Nnull	1	<56	<11
NB§ + NT _M cell culture supernatant¶	1	<56	<11
NB + Nnull + NT _M cell culture supernatant¶	23	2288	442
NB§ + Nnull cell culture supernatant¶	0	<56	<11
NB + NT _M + Nnull cell culture supernatant¶	0	<56	<11

Ig, Immunoglobulin; N, normal; NB, normal B cells; NT, normal T cells.

*The cells were cultured for 7 days in culture medium fortified with PWM. Monocytes were present in a concentration of 8% to 15%.

†The percent immunoglobulin synthesizing (cytoplasmic immunoglobulin positive) cells determined by IF.

‡Nanograms of IgM per milliliter of cell-free culture supernatant determined by ELISA.

§Cells (4×10^5) per milliliter of culture medium.

||Cells (2×10^5) per milliliter of culture medium.

¶No IgM of IgG was detected in the NT_M cell culture supernatant or in the Nnull cell culture supernatant.

findings indicate that the T, B, and null cell populations isolated by rosetting with the designated indicator cells (SRBCs, EAC, and EAG, respectively) were essentially pure in terms of their T, B, and null lymphoid cell lineage, as was reported previously.²

DISCUSSION

We have previously demonstrated that null cells are obligatory participants, in addition to T_M helper cells, monocytes, and PWM, in the synthesis and secretion of immunoglobulin by normal B cells in culture.^{1,2}

TABLE IV. The phenotypes of the circulating T cells, B cells, and null cells obtained from normal adult volunteers by rosetting procedures

MAbs or indicator cells used	Rosetted cells assayed* (% pos)		
	T cells	B cells	Null cells
MAbs			
CD2	95 ± 3	<1	<1
CD3	88 ± 4	<2	<1
CD5	93 ± 3	<1	<1
CD19	3 ± 2	70 ± 10	8 ± 4
CD20	2 ± 2	82 ± 11	6 ± 3
CD 16/56	20 ± 6	8 ± 4	60 ± 12
Indicator cells			
EAG (rosette with FcG receptor-bearing cells)	8 ± 4†	<1	>85

Pos, Positive.

*The T cells were obtained by rosetting the MNCs with SRBCs. The non-T cells were then rosetted with the EAG indicator cells to separate the null cells (which rosette with EAG) from the B cells (see MATERIALS AND METHODS). The values presented are the means and the ranges of the cells constituting the circulating T, B, and null cells obtained by rosetting in five consecutive experiments.

†These are the T_G cells (the T cells with receptors for FcG).

The cell-free supernatant of a culture of normal null cells incubated with T_M cells was able to replace the null cells in the culture.³ The active factor in this supernatant was referred to as HISFF.³ In contrast, cell-free supernatants of cultures of normal T cells, B cells, and monocytes, the cells cultured individually or in combination, were totally unable to confer to the B cells the capacity to secrete immunoglobulin, thereby confirming the null cell origin of HISFF. Since HISFF could not be replaced by the interleukins normally secreted by the T cells (IL-2 to IL-6) and the monocytes (IL-1, IL-6), it was concluded that HISFF secreted by the null cells is a mediator distinct from the interleukins heretofore considered to play a role in immunoglobulin synthesis by the B cells.³

The B cells cultured with PWM, monocytes, and either purified T_M cells or unfractionated T cells did not synthesize or secrete immunoglobulin in culture (Table III). Therefore, the failure by the B cells to synthesize and secrete immunoglobulin in the absence of the null cells cannot be attributed to the absence of a particular T cell subset.

There is still controversy surrounding the nature and legitimacy of the null lymphocytes. These cells were originally referred to as "L" (labile surface membrane immunoglobulin) cells.^{16, 17} Since these cells were devoid of the markers characteristic of T and B cells, they were referred to as the third population of cells or non-T and non-B cells.¹⁸⁻²⁰ Between the mid-1970s and the present time, these cells have also been referred to as null cells.²¹⁻²⁴ For the sake of simplicity, these cells are referred to as null cells in the text.

The T cells, B cells, and null cells isolated by differential rosetting with the SRBCs (E), EAC, and

EAG indicator cells, respectively, were essentially pure populations of lymphocytes insofar as their content of T cells, B cells, and null cells is concerned. The T cells were contaminated with very small numbers of B cells and null cells, the B cells were devoid of T and null cells, and the null cells were contaminated with a few B cells.

The significance of the CD16/CD56-positive cells within the T, B, and null cell populations must be addressed. Cells exhibiting both of these markers are considered to be NK cells. The MAb CD56 detects a surface configuration present specifically on NK cells and the MAb CD16 detects the FcG receptor that is present on NK cells and other cells as well (Terstappen L. Becton-Dickinson Immunocytometry Systems, personal communication). A significant proportion of the T cells (15% to 25%) were CD16/CD56-positive cells. These cells are the FcG receptor-bearing TG (or T_γ) CD8-positive suppressor T cells.

Most, if not all, of the CD16/CD56-positive cells in the T cell population were also CD3 positive, marking them as T cells. The small percentage of CD16/CD56-positive cells detected within the B cell population is either contaminating null cells that also carry the FcG receptor and/or NK cells. About 50% of the null cells are CD16/CD56-positive cells, but this finding is not surprising since null cells express the FcG receptor. The failure of the CD16 marker to detect all the null cells may be due to steric hindrance by IgG attached to the FcG receptor as a consequence of rosetting with the IgG antibody-sensitized EAG indicator cells and/or damage to the FcG receptors during their isolation. However, it is important to stress that the extraneous cells that contaminate the

T, B, and null cell populations isolated by rosetting procedures are present in insufficient numbers to be detected from a functional point of view.

In this investigation, we have described a humoral (antibody) immunodeficiency syndrome characterized by defective null cells in an 8 month-old male infant and a 46-year-old man. The circulating IgM, IgG, and IgA were present in exceedingly low concentrations in the circulation in both patients. The number of circulating B cells in the infant patient was markedly elevated above the normal range (approximately 10 times the normal numbers). The infant patient's B cells, cultured with autologous null cells, T cells, and monocytes, synthesized immunoglobulin but failed to secrete immunoglobulin. The percentage of the patient's B cells that synthesized immunoglobulin in culture was similar to the percentages of immunoglobulin-synthesizing B cells in cultures of age-matched normal control infants and normal adults. However, although cultured B cells obtained from age-matched infants and adults secreted significant amounts of immunoglobulin by day 7 of culture, the patient's B cells did not secrete immunoglobulin, even after 15 days of culture. These findings were observed on four different occasions in the infant patient during a period of 4 months (between 8 to 11 months of age).

The infant patient's B cells did, however, synthesize and secrete immunoglobulin by day 7 of culture after the addition of normal null cells to the cultures of the patient's MNCs at the beginning of the culture period. The amounts of IgM and IgG secreted by the infant patient's B cells were somewhat greater than the amounts secreted by the B cells of the age-matched control infants. This is probably a reflection of the greater proportion, and absolute number, of B cells in the patient's MNCs. The infant patient's B cells synthesized immunoglobulin but did not secrete immunoglobulin in culture after the addition of normal T_H helper cells, and/or normal B cells, and/or normal monocytes to the cultures of the patient's MNCs. These results indicate that the defective cell in the patient's MNCs is not a T cell, B cell, or monocyte. Furthermore, failure by the infant's B cells to secrete immunoglobulin cannot be attributed to activated autologous suppressor T cells, since the patient's B cells cultured with autologous T cells synthesized and secreted immunoglobulin after the addition of normal null cells or NHISFF to the culture. It may therefore be concluded that the infant patient's B cells are fully capable of synthesizing immunoglobulin, but they cannot secrete immunoglobulin because of the secretion by his null cells of defective HISFF or insufficient quantities of normal HISFF.

It should be noted that, in fact, the infant patient's null cells secreted a small amount of HISFF (PHISFF)

since the control normal adult's B cells cultured with normal T cells, normal monocytes, and PHISFF (NT plus NB plus NMO plus PHISFF, Fig. 2) secreted some immunoglobulin after 7 days of culture. However, the amount of immunoglobulin secreted was only 10% of that secreted by the normal adult's B cells cultured in NHISFF (NT plus NB plus NMO plus NHISFF, Fig. 2). It must therefore be concluded that the patient's null cells secreted an amount of HISFF in culture sufficient to stimulate the normal adult's B cells into limited immunoglobulin synthesis and secretion but insufficient to stimulate the infant patient's B cells to secrete immunoglobulin. Obviously, the minimum amount of HISFF required to stimulate the infant patient's B cells to secrete immunoglobulin is greater than that required to stimulate the normal adult's B cells to secrete immunoglobulin.

It must be emphasized that the null cell defect detected in the infant patient is disease related and not age related, since the B cells of six consecutive age-matched infants cultured with autologous null cells, T_M cells, and monocytes synthesized and secreted immunoglobulin in culture. Furthermore, this is not a case of physiologic hypogammaglobulinemia, a period of immunologic immaturity that normally lasts up to the age of 3 to 5 months. Since our infant patient's B cells failed to secrete immunoglobulin in vitro and in vivo, even by 11 months of age, the condition must be considered a congenital condition. Here again, it is important to note that all six randomly obtained age-matched control infants, aged 5 months to 12 months, synthesized and secreted immunoglobulin.

The number of B cells in the MNCs of the adult patient was in the normal range. However, the B cells of the adult patient cultured with autologous T cells, null cells, and monocytes, like those of the infant patient, synthesized immunoglobulin but failed to secrete significant amounts of immunoglobulin. Since these B cells, like the infant's B cells, synthesized immunoglobulin and secreted large amounts of immunoglobulin when the cultures were supplemented with normal null cells or NHISFF, it may be concluded that the defective cell is the null cell. These results suggest that the null cell defect may be congenital or acquired.

Our finding that the null cells consistently enhance immunoglobulin synthesis by the B cells irrespective of their concentration in culture with the B cells² is in agreement with the findings of Rodriguez et al.²⁵ and Lobo²⁶ who demonstrated that immunoglobulin synthesis was markedly enhanced when null cells were added to cultures of circulating MNCs in the presence of PWM.

It must be noted that the null cell defect described here to account for the immunodeficiency syndrome

in the two patients presented is not normally or uniformly found in patients presenting with the humoral immunodeficiency syndrome. We have assessed, during the past 3 years, the MNCs of five other infants and four other adults who presented with the humoral immunodeficiency syndrome, and we were unable to demonstrate a null cell defect in any of these patients.

All the findings presented are consistent with a null cell defect in the two patients. However, there appears to be at least one inconsistency in the findings reported above. Since both the synthesis and secretion of immunoglobulin by the B cells require null cells or HISFF and if the patient's null cells are defective in their capacity to secrete HISFF, how is it that the patient's B cells consistently synthesized immunoglobulin but failed to secrete immunoglobulin? The explanation is that HISFF is not a homogenous, single entity. Current investigation in our laboratory has disclosed that HISFF consists of two factors: one factor, HISFF-1, regulates or stimulates immunoglobulin synthesis by the B cells, whereas the second factor, HISFF-2, regulates or facilitates the secretion of immunoglobulin by the B cells.²⁷ It may therefore be concluded that the patient's null cells secrete HISFF-1 normally but not HISFF-2. In point of fact, the patient's null cells did secrete a small amount of HISFF-2 in addition to HISFF-1. This is indicated by the finding that the addition of PHISFF, derived from the infant patient's null cells, to cultures consisting of normal adult B cells, T_M cells, monocytes, and PWM, resulted in the normal synthesis of immunoglobulin by the B cells but resulted in the secretion of only a limited amount of immunoglobulin, approximately 10% of that normally secreted by the normal adult's B cells in the presence of NHISFF (Fig. 2). Similarly, the null cells of the adult patient secreted sufficient HISFF in culture to facilitate limited immunoglobulin synthesis and secretion by normal B cells in culture (results not presented). The small amounts of HISFF-2 secreted by the patient's null cells is obviously sufficient to facilitate the secretion of some immunoglobulin *in vivo*, thereby accounting for the low levels of IgG, IgA, and IgM in the circulation of both the infant and adult patients.

In a not insignificant percentage of patients diagnosed with the common variable hypogammaglobulinemia immunodeficiency syndrome, immunoglobulin-synthesizing B cells exist in normal numbers in the circulation but they do not or cannot secrete immunoglobulin. Immunoglobulin, especially IgG, is therefore present in the circulation in a very low concentration.²⁸⁻³⁴ Other immunologic parameters are usually present in their normal ranges. These individuals

have been referred to as nonsecretors, and it has been assumed that a defect in the B cells adversely affects their capacity to secrete immunoglobulin. On the basis of our findings that (1) null cells are obligatory participants in immunoglobulin synthesis by the B cells, (2) null cells are defective in immunodeficient patients whose B cells synthesize but do not secrete immunoglobulin (nonsecretors), and (3) the immunoglobulin-secreting capacity of the patient's B cells is restored by supplementing the cultures with normal null cells or HISFF, it would appear that the immunodeficient nonsecretors have a null cell defect(s) and not a B cell defect(s).

It must be emphasized that HISFF is distinct from any of the known interleukins considered to be involved in immunoglobulin synthesis by the B cells (IL-1, IL-2, IL-4, IL-5, and IL-6) that are all synthesized principally by T cells and monocytes. The supernatants of cultures of T cells, B cells, and monocytes, which contain all the relevant interleukins, could not replace the null cells for immunoglobulin synthesis and secretion by the B cells,³ and neither could the combination of commercially obtained IL-2, IL-3, IL-4, IL-5, and IL-6 (Cellular Products, Inc., Buffalo, N.Y.), even when they were added every second day to the cultured cells.³ Furthermore, unlike the interleukins that are secreted within 24 to 48 hours in culture, HISFF is not secreted by the null cells until day 5 or 6 of culture.³ These results are supported by results of Kimata et al.³⁵ who demonstrated that the addition of IL-1, IL-2, IL-4, and interferon- γ to their nonsecreting B cell cultures did not enhance immunoglobulin synthesis or secretion by the B cells.

Since T_M cells are required for the null cells to secrete HISFF, it could logically be speculated that HISFF is secreted by the T_M cells or the monocytes contaminating the T_M cells in response to a signal delivered by the null cells. In this investigation, we have demonstrated that the null cells secreted HISFF when they were cultured in a T_M cell culture supernatant. The latter did not contain HISFF since it did not facilitate immunoglobulin synthesis and secretion by the B cells. In contrast, the T_M cells (and their contaminating monocytes) did not secrete HISFF when they were cultured in a null cell-culture supernatant. It may therefore be unequivocally concluded that HISFF is secreted by the null cells. Since the T_M cell-culture supernatants contain factors generated by both T_M cells and contaminating monocytes, HISFF might be secreted by the null cell in response to a signal(s) secreted by the T_M cells and/or the monocytes. However, we have demonstrated that the null cells secrete HISFF in response to a signal secreted by the T_M cells²⁷ (work currently in progress).

In summary, it has been demonstrated that the humoral immunodeficiency with which our two patients presented, the ability to synthesize immunoglobulin but not to secrete immunoglobulin, can be attributed to defective null cells that are unable to secrete normal HISFF. It may be possible in the foreseeable future to correct the immunodeficiency disease caused by a null cell defect by administering purified HISFF because it is not HLA restricted.³ Current investigation is directed toward the isolation, purification, and characterization of HISFF.

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Nasal and bronchial provocation challenges with bayberry (*Myrica cerifera*) pollen extract

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*Southern bayberry, distributed throughout the southeastern United States, is the source of the fifth most common windborne tree pollen in Tampa, Fla. Fifteen percent of 400 consecutive subjects evaluated for allergic respiratory symptoms in the Tampa Bay area had positive skin tests to bayberry pollen extract. Bayberry pollen extract was obtained commercially and used to perform 25 nasal and 22 bronchial challenges on 45 subjects. Twelve of 13 (92%) study subjects with allergic rhinitis and positive skin tests to bayberry pollen extract and two of 12 (17%) control subjects with negative skin tests had positive nasal challenges ($p = 0.0001$). Four of seven (57%) study subjects with extrinsic asthma and positive skin tests to bayberry pollen extract and none of the 15 (0%) control subjects with negative skin tests had positive bronchial challenges ($p = 0.001$). Sera from 30 subjects who underwent 31 challenges were assayed for bayberry pollen extract-specific IgE by plate radioimmunoassay. Specific IgE antibodies were present in the sera of 8 of 13 (62%) subjects with positive challenges and absent in 15 of 18 (83%) subjects with negative challenges ($p = 0.01$). Intradermal skin tests with bayberry pollen extract were more predictive of provocation challenge results than plate radioimmunoassay (positive predictive value of 80%, 95% CI 62% to 98%; versus 73%, 95% CI 47% to 99%). Bayberry pollen extract is allergenic, and the Southern bayberry tree fulfills Thommen's criteria defining aeroallergen-producing plants. (*J ALLERGY CLIN IMMUNOL* 1992;90:312-8.)*

Key words: Bayberry, *Myrica cerifera*, aeroallergen, nasal provocation challenge, bronchial provocation challenge, plate radioimmunoassay (pRIA), skin test, sensitivity, specificity, predictive value

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Thirty-five tree species are in the genus *Myrica*.¹ *Myrica cerifera*, Southern bayberry (SB), is one of six species native to North America.² Other common names of *Myrica cerifera* include Southern wax myrtle, spicebush, candleberry, sweet oak, and tallow shrub. The dwarf wax myrtle, *Myrica pusilla*, is similar to, but smaller than SB, and some botanists consider them a variation of a single species.³ SB is a shrublike plant (Fig. 1) that is distributed along the Atlantic coast from New Jersey southward to Florida,