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Pathogenic anti-DNA autoantibody-inducing T helper cell lines from patients with active lupus nephritis: Isolation of $CD4^-8^-$ T helper cell lines that express the $\gamma\delta$ T-cell antigen receptor

(systemic lupus erythematosus/heat shock proteins/double-negative T cells)

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ABSTRACT **The antigen responsible for autoimmunization in systemic lupus erythematosus is unknown. In spite of this obstacle, we show that T helper (T_h) cell lines that are functionally relevant to this disease can be established *in vitro*. We derived a total of 396 interleukin 2-dependent T-cell lines from the *in vivo* activated T cells of five patients with lupus nephritis. Only 59 (≈15%) of these lines had the ability to selectively augment the production of pathogenic anti-DNA autoantibodies that were IgG in class, cationic in charge, specific for native DNA, and clonally restricted in spectrotype. Forty-nine of these autoantibody-inducing T_h lines were $CD4^+$ and expressed the $\alpha\beta$ T-cell receptor (TCR). The other 10 were $CD4^-8^-$ (double negative), 3 expressing the $\alpha\beta$ TCR and 7 expressing the $\gamma\delta$ TCR. All of the autoantibody-inducing T_h lines responded to some endogenous antigen presented by autologous B cells. The autoreactive responses of the $CD4^+$ T_h lines were restricted to HLA class II antigens, whereas those of the double-negative cells were not. Endogenous heat shock or stress proteins of the HSP60 family that were expressed by the lupus patients' B cells were involved in stimulating an autoreactive proliferation of the $\gamma\delta$ T_h cells. These studies demonstrate a novel helper activity of certain $\gamma\delta$ T cells in a spontaneous autoimmune response.**

Spontaneously produced autoantibodies that bind DNA play a major pathogenic role in systemic lupus erythematosus (SLE). However, DNA is not the inciting autoantigen (reviewed in ref. 1). Moreover, naturally produced anti-DNA antibodies are a part of the normal immune repertoire (1). Nevertheless, the pathogenic autoantibodies of SLE can be distinguished from the natural autoantibodies (2). Lupus nephritis is associated with a T helper (T_h)-cell-dependent production of the pathogenic variety of anti-DNA autoantibodies that are IgG in class and cationic in charge (2, 3). Isolation and characterization of such T_h cells may lead to an understanding of the etiologic mechanism of SLE. Although their antigenic specificities are unknown, such T_h cells can be isolated by means of their functional properties. In lupus patients, such pathogenic autoantibody-inducing T_h cells are activated *in vivo* and, therefore, they can be grown *in vitro* with low doses of interleukin 2 (IL-2) (3, 4). Here we describe 59 of these T-cell lines that had the functional ability to preferentially augment the production of pathogenic anti-DNA autoantibodies.

MATERIALS AND METHODS

Patients and T-Cell Lines. The patients with active lupus nephritis and the methods for derivation of T-cell lines from the patients' peripheral blood mononuclear cells (PBMCs) were as described (3). Helper function of the T-cell lines was determined as described (3), by quantitating the change in production of total polyclonal IgG (μg/ml) or IgG-class autoantibodies (units/ml) to single-stranded (ss) and double-stranded (ds) DNA when T cells (10^5–10^6) from the lines were cocultured with autologous B cells (10^6 per well). Cationic, IgG anti-DNA autoantibodies were detected as described (2–4). Surface phenotype of T-cell lines was determined by two-color flow cytometry (3).

T-Cell Proliferation Assays. The assays were done as described (4), except human AB serum-containing medium was used and 2×10^4 T_h-line cells were cocultured with 4×10^4 autologous antigen-presenting cells (APCs), which were either Epstein–Barr virus-transformed B-cell lines (BCLs) or PBMCs that had been either irradiated (3000 rads; 1 rad = 0.01 G_y) or fixed by glutaraldehyde (5). For all $\alpha\beta$ T cells [T cells expressing the $\alpha\beta$ heterodimeric T-cell receptor for antigen (TCR)], 3-day assays were done, and for $\gamma\delta$ T cells, 6-day assays. Cultures were incubated with [^{3}H]thymidine during the final 16 hr. In additional assays with $\gamma\delta$ T-cell lines, recombinant 65-kDa mycobacterial heat shock proteins (HSPs) that share homology with the human HSP60 family (6) were purified from bacteria expressing the plasmids pRL40 and pRL42 (gift of T. Shinnick, Centers for Disease Control, Atlanta) and added to the cultures at 0.1–100 μg/ml. To block proliferation, monoclonal antibodies (mAbs) to HLA class II (HLA-DR, -DP, and -DQ) from Becton Dickinson and mAbs to class I (HLA-A, -B, and -C) from AMAC (Westbrook, ME) were used at 8 μg/ml. N27F3-4 (IgG) (gift of W. Welch, University of California, San Francisco), a mAb that reacts with the human HSP70 family (7), was used at 1:100 dilution. IIH-9 (IgG2a) and IIIE-9 (IgG1) (from T. Shinnick), World Health Organization mAbs that react with mycobacterial 65-kDa HSP and human HSP60 (6), were used at 1:500. The anti-HSP70 mAb, N27F3-4, was supplied in ascites that contained antibodies to HSP60 produced by the mice in which the N27F3-4 hybridoma cells were grown (8). Therefore, the ascites containing N27F3-4 was used after absorption with recombinant 65-kDa HSP bound to columns, thus removing its crossreactivity to HSP60 without affecting its binding to HSP70. All mAbs were dialyzed, titrated, and added to the cultures from the beginning. For anti-HLA blocking assays the stimulator B cells (APCs) were glutaral-

Abbreviations: T_h, T helper; TCR, T-cell receptor; PBMC, peripheral blood mononuclear cell; BCL, Epstein–Barr virus-transformed B-cell line; APC, antigen-presenting cell; HSP, heat shock protein; SLE, systemic lupus erythematosus; IL-2, interleukin 2; mAb, monoclonal antibody; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA.

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Table 1. T_h cell lines that augment the production of IgG anti-DNA autoantibodies

T-cell line*	Phenotype†	IgG production, fold increase‡		
		Anti-ssDNA	Anti-dsDNA	Total
Patient JCA, 19/92 lines				
JD5	CD4	13.0	250.0	0.98
JD10	CD4	6.0	131.5	0.87
JD15	CD4	3.0	178.5	0.84
JD22	CD4	19.0	16.5	0.95
JC2	CD4	2.0	27.0	0.82
JC3	CD4	11.0	200.0	0.87
JC5	CD4	4.0	30.0	1.03
JC8	CD4	5.5	71.0	1.47
JC9	CD4	4.5	15.6	1.13
JC11	CD4	4.0	125.6	1.13
JC14	CD4	12.0	31.3	0.96
JC15	CD4	8.5	13.9	1.05
JC16	CD4	5.0	41.7	1.06
JC18	CD4	7.0	83.0	1.06
JD31	DN($\gamma\delta$)	6.0	11.0	0.85
JC4	DN($\gamma\delta$)	7.0	125.0	1.10
JC10	DN($\gamma\delta$)	6.3	19.2	1.14
JC17	DN($\gamma\delta$)	9.5	37.0	0.97
JC19	DN($\gamma\delta$)	7.5	77.0	0.95
		(0.020)	(0.010)	(17.6)
Patient MCM, 9/84 lines				
MD28	CD4	–	11.9	0.62
MD30	CD4	–	3.9	0.94
MD31	CD4	–	7.1	0.95
MD32	CD4	–	82.9	1.05
MC13	CD4	7.1	14.6	0.88
MC14	CD4	–	5.3	0.99
MC19	CD4	2.7	4.1	0.77
MC36	CD4	–	4.1	0.65
MD16	DN($\gamma\delta$)	–	7.1	0.69
		(0.010)	(0.020)	(20.4)
Patient DS-2, 5/42 lines				
DD2	CD4	7.3	5.1	1.25
DD18	CD4	7.1	2.5	0.25
DD22	CD4	5.8	2.3	0.51
DD30	DN($\alpha\beta$)	8.2	3.6	ND
AC4	DN($\gamma\delta$)	1.8	18.3	0.06
		(0.670)	(0.180)	(10.6)
Patient SE-2, 18/108 lines				
SD8	CD4	1.1	9.6	0.97
SD19	CD4	1.4	9.6	0.93
SC2	CD4	–	44.7	0.85
SC3	CD4	1.4	17.0	0.99
SC5	CD4	–	29.1	1.07
SC7	CD4	–	23.9	0.85
SC8	CD4	–	24.0	0.92
SC9	CD4	–	15.8	1.04
SC24	CD4	–	14.6	0.86
SC27	CD4	3.2	32.4	0.85
SC30	CD4	3.2	4.1	0.81
SC31	CD4	–	23.9	0.87
SC32	CD4	–	19.9	0.84
SC33	CD4	–	17.0	0.73
SC34	CD4	–	17.8	0.80
SC35	CD4	–	15.9	0.93
SD15	DN($\alpha\beta$)	1.6	11.1	0.73
SD21	CD4/DN($\alpha\beta$)	–	14.6	0.85
		(0.005)	(0.007)	(14.3)
Patient HS-2, 8/70 lines				
HD22	CD4	–	1.8	0.99
HD40	CD4	–	2.7	0.93
HD47	CD4	–	2.7	0.70
HD51	CD4	7.1	6.4	0.80
HD53	CD4	5.6	4.6	0.81
HD54	CD4	1.4	7.7	0.82
HD56	CD4	–	3.6	0.70
HD59	CD4	6.4	7.7	0.72
		(0.010)	(0.250)	(23.4)

dehyde-fixed, whereas for anti-HSP blocking, the APCs were irradiated.

Fluorescence Staining of HSP Antigens. Cells (10^5) were incubated with anti-HSP mAb followed by fluorescein-conjugated $(Fab')_2$ goat anti-mouse IgG (Fc-specific; Pel Freez Biologicals) for surface membrane or intracellular staining (3, 9). Isotype-matched mouse myeloma proteins were used as background controls with second-step reagent.

RESULTS

Only 59 of the 396 IL-2-dependent T-cell lines derived from five patients with lupus nephritis selectively augmented the production of IgG anti-DNA autoantibodies (Table 1). Forty-nine of these autoantibody-inducing T_h lines were $CD4^+$ $\alpha\beta$ TCR^+, whereas the remainder were $CD4^-8^-$ ("double negative"); of these $CD4^-8^-$ lines, 3 were $\alpha\beta$ TCR^+ and 7 were $\gamma\delta$ TCR^+ (Table 1, Fig. 1). Both types of T_h lines, $CD4^+$ or $CD4^-8^-$, grew out of limiting-dilution cultures of T cells that were initially enriched for either $CD4^+$ or $CD4^-8^-$ cells (Table 1). The autoantibody-inducing T_h lines did not increase the production of total polyclonal IgG by autologous B cells but increased the production of IgG anti-DNA autoantibodies up to 250-fold, and most of these T_h lines preferentially augmented the production of IgG autoantibodies to dsDNA. Moreover, the IgG anti-DNA autoantibodies induced by the T_h lines were cationic in charge and restricted in isoelectric pattern spectrotype (Fig. 2).

The autoantibody-inducing T_h lines were "autoreactive," proliferating in response to some endogenous antigen presented by autologous APCs (BCLs or PBMCs). The proliferative response of the $CD4^+$ $\alpha\beta$ T_h lines, in contrast to the $CD4^-8^-$ $\alpha\beta$ and $\gamma\delta$ T_h lines, could be blocked by anti-HLA-class II, and the autoreactivity of a majority of the $CD4^+$ lines tested was restricted to HLA-DR (Table 2). These $CD4^+$ T_h lines did not proliferate with HLA-mismatched BCLs from the other lupus patients (data not shown).

The autoantibody-inducing $CD4^-8^-$ $\gamma\delta$ T_h lines proliferated in response to HLA-mismatched APCs from lupus patients but not normal subjects (Table 3). The proliferative responses of the $\gamma\delta$ T_h lines were markedly greater when tested 10 days (Table 3) rather than 7 days (Tables 2 and 4) after their last feeding. The $\gamma\delta$ T_h lines could also provide HLA-nonrestricted help for production of IgG anti-dsDNA autoantibodies by B cells from unrelated lupus patients (data not shown). The autoreactive response of the $\gamma\delta$ T_h lines, unlike the $\alpha\beta$ T_h lines, could be blocked partially or completely by mAbs to the 65-kDa HSP, but the anti-HSP70 mAb had no significant effect (Table 4). However, the proliferative response of these $\gamma\delta$ T_h cells could not be augmented any further by the addition of recombinant 65-kDa HSP of

*The T-cell lines from patients with active lupus nephritis were cocultured with autologous B cells, and those lines capable of selectively augmenting production of IgG anti-DNA autoantibodies are shown. Names of T_h cell lines: first letter corresponds to that of the patient (except line AC4); second letter means that the T_h line arose from an initial limiting dilution culture of T cells that were either enriched for $CD4^+$ cells (C) or mostly depleted of $CD4^+$ and $CD8^+$ cells (D).

†All $CD4^+$ (CD4) lines were $\alpha\beta$ TCR^+. The $CD4^-8^-$, double-negative (DN) lines were either $\alpha\beta$ TCR^+ ($\alpha\beta$) or $\gamma\delta$ TCR^+ ($\gamma\delta$). One line, SD21, had two populations of T cells.

‡Values are for optimum help and represent fold increases in production of the IgG antibodies when the T cells were cocultured with autologous B cells as compared to the B cells cultured alone. ND, not determined. –, Value was below background level. Actual values for anti-DNA IgG (units/ml) and total IgG (μg/ml) produced by B cells alone are shown in parentheses.

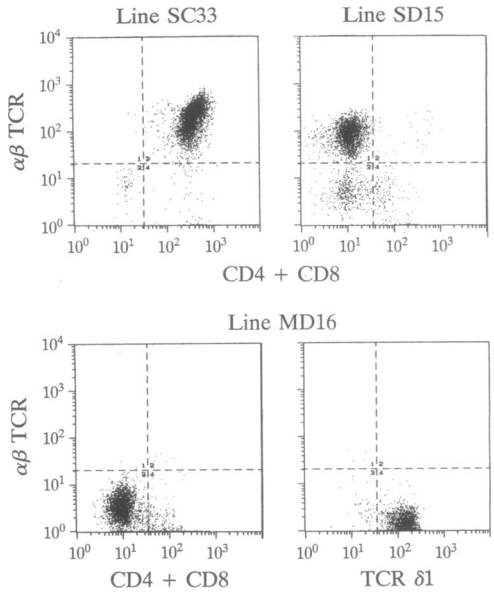

FIG. 1. Two-color immunofluorescence analysis of representative T_h lines that selectively augment production of IgG anti-DNA autoantibodies. Red fluorescence of $\alpha\beta$ TCR (WT31) staining is on *y* axis; green fluorescence of CD4 and CD8 antigens or $\gamma\delta$ TCR (TCR δ1) is on *x* axis. Based on control background staining, the dot plots were divided into quadrants to identify unstained cells (lower left), cells staining with both fluorochromes (upper right), and cells that stained with only one of the two fluorochromes (upper left and lower right). T_h line SC33 from patient SE-2 (Table 1) is $CD4^+$ $\alpha\beta$ TCR^+; line SD15 from patient SE-2 is $CD4^-8^-$ $\alpha\beta$ TCR^+, and line MD16 from patient MCM is $CD4^-8^-$ $\gamma\delta$ TCR^+. The phenotypes were stable on repeated staining.

Mycobacterium tuberculosis to the cultures containing autologous APCs (data not shown).

The various cells were stained for HSP antigens (Fig. 3). The BCLs from all five patients with active lupus expressed very high levels of HSP70 intracellularly and were 94.1 ± 2.4% (mean ± SEM) positive, whereas the corresponding values for BCLs from the three normal subjects varied, being 2% in subject 3, 35% in subject 1, and 78% in subject 2, with a markedly lower intensity of staining (Fig. 3 *B* and *C*). The B cells from the peripheral blood of all five lupus patients also

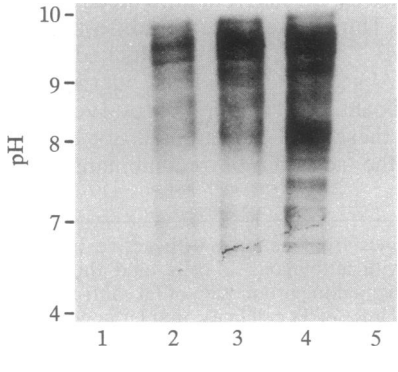

FIG. 2. Representative isoelectric focusing immunoblots of DNA-binding IgG in eluates obtained from DNA-cellulose column chromatography of supernatants from cultured lymphocytes. Lane 1, B cells of patient SE-2, cultured alone; lane 2, T_h line SC3 ($CD4^+$ $\alpha\beta$ TCR^+) from patient SE-2, cultured with autologous B cells; lane 3, T_h line SD15 ($CD4^-8^-$ $\alpha\beta$ TCR^+) from patient SE-2, cultured with autologous B cells; lane 4, T_h line JC10 ($CD4^-8^-$ $\gamma\delta$ TCR^+) from patient JCA, cultured with autologous B cells; lane 5, B cells from patient JCA, cultured alone. No antibodies were detectable in lanes 1 and 5, although these eluate samples from B cells cultured alone were concentrated 3-fold more than the eluates in the other lanes.

Table 2. HLA restriction pattern of autoreactive T_h cell lines

T-cell line	SI*	% inhibition with anti-HLA mAbs†			
		Anti-DR	Anti-DP	Anti-DQ	Anti-ABC
$CD4^+$ $\alpha\beta$					
JD22	2.5	92	8	0	0
SC24	3.0	83	0	0	4
HD40	43.3	100	0	0	0
HD47	5.5	89	18	9	12
HD51	23.8	0	0	71	0
HD53	20.9	0	75	0	0
HD56	10.5	95	0	0	0
$CD4^-8^-$ $\alpha\beta$					
SD15	2.6	9	9	1	0
SD21	4.6	0	0	0	0
$CD4^-8^-$ $\gamma\delta$					
JC4	7.4	18	0	0	26
JC10	5.5	0	0	0	20
JC17	6.1	0	0	0	0
JC19	11.2	0	16	0	0
JD31	6.4	0	36	0	0

*In this and subsequent tables, the proliferative responses of representative autoantibody-inducing T_h lines to autologous BCLs are expressed as stimulation index (SI). SI was calculated by subtracting the mean cpm incorporated by BCL cultured alone from that incorporated by the BCL/T_h coculture and then dividing that value by the mean cpm incorporated by T_h cells cultured alone. [^{3}H]Thymidine incorporation by T_h cells cultured alone ranged between 190 and 426 cpm and that by the glutaraldehyde-fixed BCLs ranged between 195 and 511 cpm. All cultures were set up in triplicate and the data are representative of three experiments for each T_h line. SDs were <10% of the mean values.

†In this and subsequent tables, the percent inhibition of the proliferative response equals (1 − {[(mean cpm T_h cells plus BCL) − (mean cpm BCL alone) in presence of mAb]/[(mean cpm T_h cells plus BCL) − (mean cpm BCL alone) in absence of mAb]} × 100. A value of 0 means no inhibition. Isotype-matched myeloma proteins did not inhibit the proliferative responses (data not shown). Similar results were obtained with autologous PBMCs as stimulator cells (data not shown).

expressed high levels of HSP70 intracellularly, being 19.6 ± 2.2% positive. By contrast, HSP70 was barely detectable in the peripheral B cells of four normal subjects tested (0.47 ± 0.14% positive). The autoantibody-inducing T_h lines from the lupus patients were also strongly positive for intracellular HSP70 (70–90%). T-cell lines from normal subjects were also positive (30–50%), but with a lesser intensity (Fig. 3*H*). Similar results were obtained upon staining the various cell populations with the mAbs to the HSP60 family, but the staining was more punctate (not shown). Despite several attempts, we could not detect HSP antigens on the surface of the lupus B cells or the various cell lines.

DISCUSSION

Only ≈15% of the T-cell lines derived from the five patients with active lupus nephritis were able to selectively augment IgG anti-DNA autoantibody production. Therefore, help is not provided indiscriminately by any T cell that is activated in lupus patients. Isolated B cells from the lupus patients spontaneously produced high levels of total IgG in culture, a sign of polyclonal B-cell hyperactivity (1). However, the autoantibody-inducing T_h lines that we selected did not increase the production of total polyclonal IgG any further but did increase the production of IgG anti-DNA autoantibodies up to 250-fold. The increase in IgG anti-DNA antibodies induced by the T_h lines was not reflected by an increase in total IgG, since the autoantibodies constituted a small component of the total IgG produced in the cultures. Most of the T_h lines preferentially increased the production

of IgG autoantibodies to dsDNA, which are associated with the development of lupus nephritis in humans (1). Therefore, these T_h lines interacted specifically with those B cells in the culture that were committed to produce pathogenic, IgG-class anti-DNA autoantibodies.

Most of the pathogenic autoantibody-inducing T_h lines were $CD4^+$ $\alpha\beta$ TCR^+, and their autoreactivity could be blocked by anti-HLA-D mAbs, indicating a cognate interaction between their TCRs and the HLA–autoantigen complex. A majority of these $CD4^+$ T_h lines were restricted to HLA-DR. In murine lupus, not all "autoreactive" T_h cells can induce the pathogenic variety of anti-DNA autoantibodies (4), indicating that the autoantibody-inducing T_h cells recognize some endogenous peptide in addition to class II molecules.

Only 3 of the 59 human T_h lines were $CD4^-8^-$ and $\alpha\beta$ TCR^+, although T_h cells of this unusual phenotype are markedly expanded in lupus patients (3). Perhaps the culture conditions were not optimal for these cells or they might prefer to grow together with the $CD4^+$ T_h cells (Table 1, line SD21). Nevertheless, the $CD4^-8^-$ $\alpha\beta$ T_h lines (Table 1, Fig. 1) have maintained a stable $CD4^-8^-$ phenotype for over a year in culture. These $\alpha\beta$ TCR^+ double-negative cells might have high-affinity receptors for self antigens, but their progenitors might have escaped deletion in the thymus due to a failure to express CD4 and/or CD8 (10). Indeed, double-negative, pathogenic autoantibody-inducing T_h cells expressing "forbidden" or autoreactive TCRs can also be found in the periphery of non-*lpr* lupus mice (11), and they are distinct from those found in MRL-*lpr*/*lpr* mice, since the latter do not help or express the forbidden autoreactive TCRs (11).

Table 3. HLA-nonrestricted proliferation of autoantibody-inducing $CD4^-8^-$ $\gamma\delta$ T_h cell lines

Source of APCs* (B cells)	Proliferative response of T_h line, SI†				
	JC4	JC10	JC17	JC19	JD31
BCLs					
JCA	26.3	19.5	6.8	34.2	57.1
MCM	30.4	47.9	<1.0	4.6	6.6
DS-2	48.1	47.8	71.4	12.5	11.7
SE-2	41.8	57.9	21.2	7.2	6.5
HS-2	10.5	15.4	15.8	16.2	25.7
Normal 1	<1.0			1.8	
Normal 2	<1.0	<1.0	2.3	3.7	1.5
PBMCs					
JCA	12.7			11.5	
MCM	20.1			7.6	
DS-2	2.7			18.1	
HS-2	<1.0			13.2	
Normal 7	<1.0			<1.0	
Normal 11	<1.0				
Normal 17	2.8				

*Lupus patients and normal subjects were HLA-typed at the National Institutes of Health Blood Bank as follows.

Lupus patients

JCA:	A31,− ;	B18,35;	Cw3,w4;	DR2,9w53 ; DQ1,2
MCM:	A23,29;	B18,− ;	Cw2 ;	DR1,5,w52 ; DQ1,3
DS-2:	A11,28;	B8,35 ;	Cw4 ;	DR1,3,w52 ; DQw1,2
SE-2:	A1,11 ;	B8,− ;	Cw7,− ;	DR3,−,w52; DQw2
HS-2:	A29,31;	B15,40;	Cw3 ;	DR2,4,w53 ; DQ1,3

Normal subjects

No. 17:	A2,29;	B7,44 ;	Cw7;	DR2,w− ; DQw1
No. 11:	A2,29;	B7,44 ;	C5,6;	DR6,w52 ; DQw1
No. 7 :	A2,− ;	B18,51;	Cw1;	DR2,5,w52; DQ1,3

†See Table 2. The mean cpm incorporated by the T_h-line cells cultured alone ranged between 100 and 222, and that by the irradiated BCLs from lupus patients (lupus) and normal subjects ranged between 244 and 5446. The mean cpm incorporated by irradiated peripheral blood B cells from the lupus patients ranged between 50 and 106, and that by the B cells of normal subjects between 171 and 435.

Table 4. Anti-HSP mAbs block the autoreactive proliferation of $\gamma\delta$ T_h cell lines

T-cell line	SI*	% inhibition by anti-HSP mAb†		
		IIH-9	IIIE-9	N27F3-4
$CD4^-8^-$ $\gamma\delta$				
JC4	7.4	100	47	0
JC10	4.5	100	73	3
JC17	4.4	46	63	26
JC19	8.4	52	31	0
JD31	3.9	48	0	0
$CD4^-8^-$ $\alpha\beta$				
SD15	5.1	0	25	1
SD21	3.2	0	0	0
$CD4^+$ $\alpha\beta$				
HD47	3.6	0	0	0
HD56	10.5	0	0	0
SC2	2.9	10	13	0
SD19	9.2	0	0	0
JD5	9.7	0	3	0
JC18	8.6	3	ND‡	0

*See Table 2. Representative autoantibody-inducing T_h cell lines are shown. Irradiated autologous BCLs were used as stimulator cells, and the mean cpm incorporated by these irradiated cells cultured alone ranged between 2704 and 8266.

†See Table 2. Data are representative of three experiments done with each T_h line. Similar results were obtained when irradiated, autologous PBMCs were used as stimulators (data not shown).

‡Not done.

Of major interest are the $CD4^-8^-$ $\gamma\delta$ lines that augmented the production of pathogenic anti-DNA autoantibodies, in one case up to 125-fold (Table 1, line JC4). All of these $\gamma\delta$ T_h lines failed to stain with Leu-7 and Leu-11b (CD16) mAbs for natural killer cells (data not shown). Previously, we had not detected an expansion of these cells in the peripheral blood of lupus patients (3). However, since a purified $CD3^+4^-8^-$ T-cell subpopulation from active lupus could augment the production of pathogenic autoantibodies (2, 3), the $CD4^-8^-$ $\gamma\delta$ cells contained in that population could have helped along with the $CD4^-8^-$ $\alpha\beta$ cells. T cells expressing the $\gamma\delta$ TCR are known to manifest cytolytic function, and a subset of these cells is specific for mycobacterial antigens, particularly HSPs (12–18). However, the widely conserved HSPs (stress proteins) may also be expressed by autologous cells (19). Indeed, the mAbs to mycobacterial 65-kDa HSP that blocked the autoreactive proliferation of the $\gamma\delta$ T_h cells are known to crossreact with determinants shared by the human HSP60 family (6, 8, 19, 20). The HSP families of stress proteins are expressed by activated B cells and macrophages (8, 21) and they may act as molecular "chaperones" for processed antigens presented by these cells (22). The pathogenic autoantibody-inducing $\gamma\delta$ T_h cells described here were probably responding to HSP60-associated autoantigens expressed by the activated lupus B cells; however, their proliferative responses could not be augmented any further by addition of mycobacterial 65-kDa HSP antigens that share homology with human HSP60. In this respect, these lupus $\gamma\delta$ T_h cells are different from the previously described $\gamma\delta$ cells (13–18), which were deliberately primed to mycobacterial antigens in most cases. Perhaps these lupus $\gamma\delta$ T_h cells were responding maximally to the HSP60-related antigens expressed by autologous activated B cells, and therefore addition of exogenous HSP60 did not have any further effect. Since HSPs may be intimately involved in antigen presentation (8, 22), the anti-HSP60 mAbs could have inhibited proliferation of this subset of $\gamma\delta$ T cells by sterically inhibiting presentation of some HSP-associated autoantigen. Indeed, the $\gamma\delta$ T_h lines

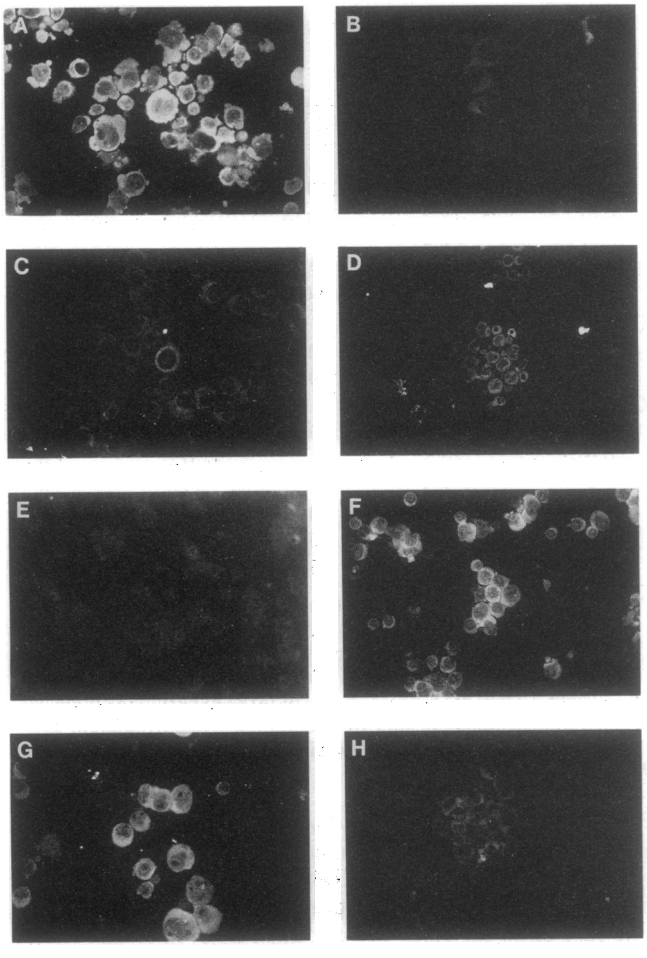

FIG. 3. Representative examples of intracellular staining of HSP antigens by the N27F3-4 mAb. (*A*) BCL derived from patient MCM with active lupus. (*B* and *C*) BCLs from normal subjects 1 and 2. (*D*) Peripheral blood B cells from lupus patient MCM. (*E*) peripheral blood B cells from a normal subject 4. (*F*) Autoantibody-inducing $CD4^-8^-$ $\gamma\delta$ T_h line JC4 from lupus patient JCA. (*G*) Autoantibody-inducing $CD4^+$ $\alpha\beta$ T_h line JD5 from patient JCA. (*H*) $CD8^+$ $\alpha\beta$ T-cell line from a normal subject.

proliferated in response to B cells from unrelated lupus patients but not in response to HSP-positive BCLs from normal subjects. The lupus B cells, unlike the normal B cells, could be presenting a crossreactive idiotypic determinant shared by the pathogenic anti-DNA autoantibodies, in association with HSP60, to these select $\gamma\delta$ T_h lines. The marked increase in HSP within the lupus B cells also suggests that those B cells were producing some aberrant protein molecules that were improperly folded (19). The anti-HSP mAbs did not inhibit the proliferative responses of the $\alpha\beta$ T_h lines. Therefore, the anti-HSP mAbs were not exerting a nonspecific toxic effect and perhaps the T_h lines, unlike the lupus B cells, expressed only intracellular HSP. Indeed, activated T cells can express HSP intracellularly (23), whereas activated B cells and macrophages may express HSP on their surface (8, 22). We did not detect HSP antigens on the surface of the lupus B cells by immunofluorescence, probably due to a transient expression of these proteins on their membranes. The autoreactive responses of the $\gamma\delta$ T_h cells described here may not be restricted to any HLA or class I-like molecules. Even CD1 restriction is found very infrequently among $\gamma\delta$ T-cell lines (12). Moreover, CD1 is not expressed by BCLs (24), which did stimulate these $\gamma\delta$ T_h lines. In mice, $\gamma\delta$ T cells, in concert with other T cells, were shown to proliferate upon immunization with syngeneic B-lymphoma cell lines and augment immunoglobulin production by those lymphomas (25). However, the $\gamma\delta$ T_h cells we selected are participants in a spontaneous autoimmune response, and they help autologous B cells that produce pathogenic autoantibodies. It is intriguing that patients with SLE spontaneously produce autoantibodies to HSP (26) and $\gamma\delta$ T cells responding to HSP60 are found in the joints of patients with rheumatoid arthritis (15), another rheumatic autoimmune disease.

The IgG anti-DNA autoantibodies induced by the T_h lines were cationic in charge and restricted in spectrotype (Fig. 2), indicating that a select B-cell population produced these pathogenic autoantibodies. A cognate interaction may have occurred between the pathogenic autoantibody-producing B cells and the three types of T_h cells described here, because this select B-cell population was not indiscriminately helped by all activated T-cell lines from lupus patients. Moreover, the T_h lines that we selected augmented preferentially the production of pathogenic autoantibodies, not polyclonal IgG. Further characterization of the structures and specificities of the TCRs expressed by these functionally defined T_h cells could lead to an understanding of the etiologic mechanism of SLE.

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