SUPPRESSOR-CELL INDUCTION IN VITRO

IV. Target of Antigen-Specific Suppressor Factor and Its Genetic Relationships*

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Suppressor cells (SC)[1] are now recognized to be important in the regulation of immune responses of many, and perhaps all types (1–13). Both antigen-specific and nonspecific SC have been reported (1–13, 14–17). In the majority of the reports, these are T cells of the Ly 2^+ 3^+ phenotype (9, 10, 12, 13, 18, 19), and are usually Ia^+ (9, 18, 19). We have been interested in studying antigen-specific suppressor cells induced in vitro, because of the relative ease of investigation of both the mechanisms of induction, and of suppression with these cells (5, 20, 21). Culturing T cells from spleen with high concentrations of either proteins such as keyhole limpet hemocyanin (KLH), chicken gamma globulin, or synthetic polypeptide antigens such as (T, G)-A–L or GAT^{10} (5, 20–22) yields antigen-specific suppressor cells with the Ly 1^- 2^+ 3^+ Ia^+ phenotype (18). These SC are derived from an interaction of two T cells; the SC amplifier (Ly 1^+ 2^+ 3^+ Ia^-) and SC precursor (Ly 1^- 2^+ 3^+ Ia^-) (23). In vitro-induced SC function efficiently in vitro in very small numbers, with 10^3 cells often totally suppressing primary immune responses. This high efficiency of the SC effect prompted us to wonder whether the action of SC in vitro required contact with the appropriate target cell, or whether supernatant factors were released which mediated their effect. Culture of SC with antigen for 24 h yielded supernates, termed suppressive factor (SF), which mediated specific suppression (21). Because other workers have not detected SF in supernates of SC of antibody responses induced in vivo (24), it was essential to prove that SF were released from viable cells, and were not the sheddings from dead cells. This was demonstrated by the use of the metabolic inhibitor, sodium azide, which abolished the release of SF from SC (21).

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[1] *Abbreviations used in this paper:* AFC, antibody-forming cells; ALS, antilymphocytic serum; ATX, adult thymectomy; C′, complement; DNP, 2,4-dinitrophenyl determinant; GAT^{10}, random copolymer of 60% glutamic acid, 10% alanine and 10% tyrosine; HC, helper cells; HF, helper factor(s); KLH, keyhole limpet hemocyanin; PAA, polyacrylamide beads; SC, suppressor cells; SE, supernate of sonicated in vivo-induced suppressor cells; SF, suppressor factors; TNP, 2,4,6-trinitrophenyl determinant.

The mechanism by which SF acts is not known in detail. Because of the antigen (carrier) specificity of the suppressor effect which was not expressed on thymus-independent responses, it was known that SF acted at some step in either helper-cell induction or function. The studies reported here were designed to localize the cellular target of SF. Because there have been many reports of the effects of extracts from in vivo-induced suppressor cells (SE) on the antibody response (24–29), we have compared our results involving SF with those obtained with extracts, and we have found a marked disparity, indicating that SF does not act like SE.

Materials and Methods

Mice. CBA/Ca, C57Bl/10ScSn(B10), C57Bl/ICRF, (CBA × B10)F_1, and A/Sn mice bred at the Imperial Cancer Research Fund Breeding Unit, or B10 (= C57 Bl/10) and B10 congenic mice B10.Br, B10.A (4R), B10.A (5R), B10.HTT, B10.A, and SJL and (CBA × B10)F_1 mice from the Clinical Research Centre, Harrow, Middlesex, England were used, as well as CBA/Ca B10.A and A/J mice from the Department of Bacteriology and Immunology, University of Helsinki.

Antigens. KLH used was either donated by Dr. Marvin Rittenberg, Portland, Ore. or purchased from Calbiochem, San Diego, Calif. It was used in a concentration of 0.1 μg/ml for in vitro induction of helper cells, and in a concentration of 100 μg/ml for in vitro induction of suppressor cells. Trinitrophenylated (TNP) KLH (TNP-KLH; eight groups of TNP/100,000 daltons), kindly prepared by Dr. Marilyn Baltz in our laboratory in London, was used in a concentration of 0.1 μg/ml for in vitro cooperation between in vitro KLH-primed helper cells and normal spleen cells, or in a concentration of 0.01 μg/ml to trigger secondary in vitro responses by in vivo TNP-KLH-primed (B10 × CBA)F_1 or CBA mice. For in vivo priming, TNP-KLH was coupled to bentonite by the method of Rittenberg and Pratt (30), and was used as described previously. As a control, SF from an in vitro copolymer of L-glutamic acid,-L-alanine,-L-tyrosine (GAT^{10}; a gift from Dr. Paul Maurer, Philadelphia)-primed suppressor cells (priming dose 100 μg of GAT^{10}/ml, 1 μg/ml for the production of SF) were used. To test T-independent B-cell responses, thymus-independent antigens 2,4-dinitrophenyl (DNP)-Ficoll (0.1 μg/ml) or 0.3% DNP-polyacrylamide beads (PAA) were used. They were donated by Professor O. Makela, Helsinki and Dr. D. Parker, London.

In Vitro Cultures. Marbrook–Diener cultures incubated in an atmosphere of 10% CO_2 in air in a humidified incubator at 37°C were used throughout. The conditions used for the induction of helper cells (HC) specific for KLH (HC_{KLH}) or SC_{KLH} were as originally reported (5, 31, 32). SF_{KLH} were produced as described previously (21). The helper supernates were produced in a similar way, but using HC_{KLH} as a cell source. These procedures are summarized in Table I. The usual cooperative cultures contained 3×10^5 HC_{KLH} and 15×10^6 normal spleen cells stimulated with TNP-KLH, and they were cultured for 4 days. Suppression was induced by adding SF to the above mixture, and it was measured by the decrease in the cooperative response. The cooperative culture protocols are summarized in Table II. For secondary in vitro responses, $10–15 \times 10^6$ in vivo TNP-KLH-primed spleen cells were cultured with TNP-KLH with or without SF in Marbrook-Diener flasks for 5–6 days.

Treatment of Mice. Adult thymectomy (ATX) was performed by the method of Miller (33) on 6-wk-old mice. These mice were used 2–3 mo later; 48 h before the removal of spleens they were injected with 0.5 ml of anti-lymphocyte serum (ALS) kindly donated by Dr. Elizabeth Simpson at the Clinical Research Centre. Half of the dose was given intraperitoneally, half subcutaneously. The spleens of these mice are referred to as ATX + ALS spleens. To induce peritoneal exudate cells, mice were injected intraperitoneally with 2 ml of 2% starch in phosphate-buffered saline 4 days before the harvest of exudate cells using saline lavage of the peritoneal cavity. To get macrophage monolayers, $2–5 \times 10^6$ peritoneal exudate cells were plated on 3.5-cm diameter Petri dishes, cultured for 2 days, washed, and then used for absorptions.

Treatment of Cells. In vitro-induced HC were passed through nylon wool columns (passed cell yields of 60–80%) (34) or subjected to the treatment with anti-Ly 2.1 antiserum (kindly donated by Dr. I. F. C. McKenzie, Melbourne, and retitrated with our rabbit complement by Dr. P. C. Beverley in our laboratory in London) and rabbit complement as described earlier (35).

TABLE I
Production of SF In Vitro

First Culture	Second Culture	SF
15×10^6 normal spleen cells + 100 μg of KLH/ml for 4 days in vitro	5×10^6 washed, viable cells from first culture + 0.1 μg of KLH/ml for 24 h in vitro	Supernate of the second culture after 1,000 rpm × 10 min spin filter if necessary

TABLE II
Assay of Help and Suppression

Stimulus		Suppression	Response of spleen cells (Antibody-forming cells/culture)
HC	Antigen		
–	TNP-KLH	–	Background
HC_{KLH}	TNP-KLH	–	Help
HC_{KLH}	TNP-KLH	SF_{KLH}	Suppression

An additional group of spleen cells containing the optimal concentration of a thymus-independent antigen, DNP-Ficoll and DNP-polyacrylamide, was often included to measure B-cell responses.
% Response = (suppression − background)/(help − background) × 100.

Normal spleen cells and peritoneal exudate cells were treated with anti-Thy-1.2 (antiserum prepared by Dr. Elizabeth Simpson and tested by Dr. P. C. Beverley) and rabbit complement as described (35). Normal spleen cells were also passed through nylon wool columns (25–30% passed cell yields) (34).

Assay of Antibody-Forming Cells (AFC). The responses were evaluated by assaying the number of IgM and IgG (using goat anti-μ and sheep anti-IgG enhancing sera) anti-DNP (dinitrophenyl, cross-reacting hapten to TNP) (36) AFC/culture (31). The results are expressed as means of triplicate cultures ± standard error.

Results

Action of SF on Helper Pathway. The carrier specificity of the action of SF, as well as its lack of effect in thymus-independent B-cell responses suggested that the functional target of SF is at some stage in the helper pathway (21). It is possible to further dissect this stage by comparing the effect of SF on cultures stimulated by helper factors (HF) or HC. If HC are used, SF has a marked effect (21). There is none, however, if HF is used (Table III, Howie et al. unpublished observations). Thus, the effect of SF is at a step before HC release HF—on differentiated HC, or on the cells involved in HC induction (helper cell precursors/amplifiers or macrophages).

Action of SF on Nylon Wool-Passaged Helper Cells. After 4 days, the in vitro cultures used to induce helper cells still contain other non-helper cell types, such as macrophages and residual B cells, in addition to T cells. To further clarify the T-cell nature of the target of SF, the in vitro induced HC_{KLH}, after induction, were passaged through nylon wool columns known to bind macrophages, B cells, and many T cells, chiefly those with the Ly 1^+ 2^+ 3^+ phenotype (37). As shown in Table IV, equally effective suppression was obtained when either unpassaged HC or nylon wool-passaged HC were used. Thus, the SF eventually acts on a T cell in the helper T-cell pool which is not adherent to nylon wool. However, SF could act first on an auxillary T cell in

TABLE III
Lack of Effect of SF on HF-Stimulated Responses

Stimulus		Suppression	Response (Anti DNP-AFC/ culture ± standard error)
HC/HF	Antigen		
–	TNP-KLH	–	50 ± 25
CBA HC_{KLH}	TNP-KLH	–	677 ± 13
	TNP-KLH	CBA SF_{KLH} 10%	130 ± 12
CBA HF_{KLH} 0.1%	TNP-KLH	–	257 ± 79
	TNP-KLH	CBA SF_{KLH} 10%	273 ± 46
	TNP-KLH	CBA SF_{KLH} 1%	363 ± 60

SF_{KLH} produced by KLH-specific suppressor cells of CBA origin in vitro was added to the mixture of in vitro-induced HC_{KLH} or CBA HF_{KLH} and normal spleen cells, the mixture was challenged with TNP-KLH, and the numbers of anti-DNP antibody-forming cells were assayed after 4 days of culture in vitro. Five other experiments gave analogous results.

TABLE IV
Action of SF on Nylon Wool-Passed HC

Stimulus		Suppression	Anti-DNP-AFC/ culture ± standard error	Response
HC	Antigen			
				%
–	DNP-PAA	–	3,316 ± 248	–
–	TNP-KLH	–	170 ± 38	0
CBA HC_{KLH}	TNP-KLH	–	1,343 ± 110	100
CBA HC_{KLH}	TNP-KLH	B10.HTT SF_{KLH} 10%	355 ± 105	16
CBA HC_{KLH}	TNP-KLH	B10.A SF_{KLH} 10%	630 ± 26	39
CBA HC_{KLH} (pNW)*	TNP-KLH	–	1,207 ± 330	100
CBA HC_{KLH} (pNW)	TNP-KLH	B10.HTT SF_{KLH} 10%	300 ± 105	13
CBA HC_{KLH} (pNW)	TNP-KLH	B10.A SF_{KLH} 10%	410 ± 64	23

SF_{KLH} at a final concentration of 10% of either B10.HTT or B10.A origin was added to the mixture of CBA in vitro-induced HC_{KLH} either untreated or filtered through nylon wool (pNW), and normal CBA spleen cells. The mixture was challenged with TNP-KLH, and the numbers of anti-DNP antibody-forming cells assayed after 4 days of culture in vitro.
Five other experiments gave analogous results.
* pNW – passed through nylon wool.

normal spleen. To exclude this possibility, nylon wool-passaged HC were cultured with anti-Thy-1 and complement-treated spleen cells. Suppression was unaffected, suggesting that the target for the action of SF was within the nylon wool-passed cell population (data not shown).

Ly-2$^+$ 3$^+$ or Ly-1$^+$ 2$^+$ 3$^+$ Cells Are Not the Target of SF. Primed T-cell populations contain cells with different Ly phenotypes. In addition to Ly 1$^+$ 2$^-$ 3$^-$ cells, and helper effector cells (18), there are cells of the Ly 1$^+$ 2$^+$ 3$^+$ Ia$^-$ phenotype (e.g. amplifier cells in helper/suppressor cell induction, [23]) and of the phenotype Ly 1$^-$ 2$^+$ 3$^+$ Ia$^+$ (e.g. suppressor effector cells [23]). To further clarify the characteristics of the actual target cells for the action of SF, HC populations were treated with anti-Ly-2 antisera and complement (C′). This

procedure did not affect suppression, suggesting that primed Ly-2-positive cells (e.g. either Ly 1^+ 2^+ 3^+ or Ly 1^- 2^+ 3^+ cells) were not required for the function of SF (Table V). To exclude the possibility that unprimed Ly 2^+ 3^+ or Ly 1^+ 2^+ 3^+ cells present in normal spleen could act as an Ly 2^+ or Ly 1^+ 2^+ mediator of the SF effect anti-Ly-2 + C′-treated HC were cultured with anti-Thy-1 + C′-treated spleen cells. These procedures did not abolish suppression (Table VI). These results suggest that Ly 2^+ cells (either Ly 2^+ 3^+ or Ly 1^+ 2^+ 3^+) are not essential for the function of SF.

Receptors for SF on Other Cell Types. In addition to the functional target cell of SF present in nylon wool-passed HC, and insensitive to anti-Ly 2.1 serum + C′, other cell types might carry a receptor for the SF. In comparison to HC, other cell types proved inefficient in absorbing out the activity of SF. Thus syngeneic spleen cells, splenic T cells, or ATX + ALS spleen cells, a population consisting chiefly of B cells and macrophages, did not absorb out the activity of SF. This is in contrast with nylon wool-passed HC, which on cell-to-cell basis were much more efficient than untreated HC. The efficiency of HC in absorbing out the activity of SF is in contrast to the in vitro-induced SC, which did not absorb out the SF activity. However, syngeneic peritoneal exudate cells did absorb out some of the SF activity. This effect was lost, however, when macrophage monolayers derived from peritoneal exudate cells rather than peritoneal exudate cells as such were used for absorption (Table VII). Other activated T cells (in addition to HC and SC) have been tested for their ability to absorb out the activity of SF. These results will be reported elsewhere.

Lack of Genetic Restriction in the Action of SF. In the in vitro culture system used in these experiments, products controlled by the *I-A* subregion of the *H-2* complex have been shown to be involved in the triggering of helper cells by specific macrophage products (38, 39), and as being part of the helper factor molecule (40). Therefore, it was of interest to determine whether I-controlled products were involved in the function of SF and whether genetic restriction was expressed at a functional level as in the macrophage T-cell interaction (38). When SF_{KLH} produced in B10, B10.A, B10.A(4R), B10.A(5R), B10.Br, or B10.HTT and in A/Sn (or A/J) or SJL mice were tested for their ability to suppress TNP-KLH responses by CBA HC-KLH or in secondary TNP-KLH responses by in vivo TNP-KLH-primed (CBA × B10)F_1 or CBA spleen cells, the suppression was equal in magnitude to that caused by syngeneic SF_{KLH}. In the secondary response to TNP-KLH, both IgM and IgG were suppressed to the same extent. Also, when HC_{KLH} of B10 or C57Bl origin were used as a target for SF, equal suppression was obtained (Tables IV–VI, VIII, IX).

These results differ from the results of Taniguchi et al. using suppressive extracts of in vivo-induced suppressor cells, where certain strains such as A/J do not produce suppressive extracts, and other strains, such as B10 and the B10 congenics, are nonsuppressible (26). Also, the genetic requirements for effective suppression differ, since in the system of Taniguchi et al., *I-J*-region homology is required between the strains yielding suppressor cell extract and the target for effective suppression (26).

TABLE V

Lack of Requirement of Primed Ly 2⁺ or Ly 1⁺ 2⁺ Cells for the Action of SF

Stimulus		Suppression	Anti-DNP-AFC/ culture ± standard error	Response
HC	Antigen			
				%
–	DNP-PAA	–	2,523 ± 375	–
–	TNP-KLH	–	137 ± 62	–
CBA HC_{KLH}	TNP-KLH	–	740 ± 159	100
CBA HC_{KLH}	TNP-KLH	CBA SF_{KLH} 10%	93 ± 93	<0
CBA HC_{KLH}	TNP-KLH	B10.HTT SF_{KLH} 10%	317 ± 116	30
CBA HC_{KLH} (α-Ly 2.1 + C′)	TNP-KLH		730 ± 136	100
CBA HC_{KLH} (α-Ly 2.1 + C′)	TNP-KLH	CBA SF_{KLH} 10%	167 ± 27	5
CBA HC_{KLH} (α-Ly 2.1 + C′)	TNP-KLH	B10.HTT SF_{KLH} 10%	136 ± 27	0
CBA HC_{KLH} (C′)	TNP-KLH	–	640 ± 57	100
CBA HC_{KLH} (C′)	TNP-KLH	CBA SF_{KLH} 10%	130 ± 54	<0
CBA HC_{KLH} (C′)	TNP-KLH	B10.HTT SF_{KLH} 10%	140 ± 85	0

SF_{KLH} at a final concentration of 10% of either CBA or B10.HTT origin was added to the mixture of CBA in vitro-induced HC_{KLH} either untreated or treated with anti-Ly-2 antiserum and C′ or C′ alone and normal CBA spleen cells. The mixture was challenged with TNP-KLH, and the number of anti-DNP antibody-forming cells was assayed after 4 days culture in vitro. The anti-Ly-2 serum used was tested for serological specificity on B10 and B6-Ly 2.1. 3.1, and functionally on SC and HC. It did not lyse B10 (Ly 2.2) lymph node cells in the presence of C′, and did not effect the function of HC. Two other experiments gave analogous results.

TABLE VI

Lack of Requirement of Nonprimed Ly 2⁺ or Ly 1⁺ 2⁺ Cells for the Action of SF

Stimulus			Suppression	Anti-DNP-AFC/ culture ± standard error	Response
HC	Antigen	Spleen			
					%
–	DNP-PAA	Normal	–	1,056 ± 256	–
–	TNP-KLH	Normal	–	50 ± 21	0
CBA HC_{KLH}	TNP-KLH	Normal		263 ± 37	100
CBA HC_{KLH}	TNP-KLH	Normal	CBA SF_{KLH} 10%	83 ± 3	15
CBA HC_{KLH}	TNP-KLH	Normal	B10.HTT SF_{KLH} 10%	60 ± 15	5
CBA HC_{KLH} (C′)	TNP-KLH	Normal	–	323 ± 12	>100
CBA HC_{KLH} (α-Ly 2.1 + C′)	TNP-KLH	Normal	–	223 ± 27	100
CBA HC_{KLH} (α-Ly 2.1 + C′)	TNP-KLH	Normal	CBA SF_{KLH} 10%	30 ± 12	<0
CBA HC_{KLH} (α-Ly 2.1 + C′)	TNP-KLH	Normal	B10.HTT SF_{KLH} 10%	43 ± 17	<0
CBA HC_{KLH} (α-Ly 2.1 + C′)	TNP-KLH	α-Thy-1 + C′	–	210 ± 53	100
CBA HC_{KLH} (α-Ly 2.1 + C′)	TNP-KLH	α-Thy-1 + C′	CBA SF_{KLH} 10%	30 ± 17	<0
CBA HC_{KLH} (α-Ly 2.1 + C′)	TNP-KLH	α-Thy-1 + C′	B10.HTT SF_{KLH} 10%	23 ± 12	<0

SF_{KLH} at a final concentration of 10% of either CBA or B10.HTT origin was added to the mixture of in vitro CBA-induced HC_{KLH}, either untreated or treated with anti-Ly-2 antiserum and C′ or C′ alone, and normal or anti-Thy 1.2 plus C′-treated CBA spleen cells. The mixture was challenged with TNP-KLH and the number of anti-DNP antibody-forming cells was assayed after 4 days culture in vitro. Anti-Thy 1.1 plus C′ treatment abolished the response to sheep erythrocytes, but left the response to DNP-PAA unaltered. One other experiment gave analogous results.

Discussion

Because of the increasing number of reports of the importance of various types of SC in regulating immune responses, there has been much interest in the mechanism by which suppressor cells exert their effects. The obvious questions which have been raised include whether or not the mechanism of suppression involves cell-to-cell contact with the suppressible cell, whether or not it involves diffusible mediators, and what the nature of the latter may be. Using antigen-specific suppressor cells induced in vivo, Tada et al. (2), Kapp et al. (27, 28), and Theze et al. (29) have isolated an extract which is a putative mediator of suppression, and they have described its mode of action. Because of

TABLE VII
Absorptions of SF by Lymphoid Cells

Experiment	Stimulus		Suppression		Anti-DNP-AFC/culture ± standard error	Response
	HC	Antigen	SF	Absorptions		
			10%			%
I	–	TNP-KLH	–	–	30 ± 6	0
	CBA HC_{KLH}	TNP-KLH	–	–	397 ± 78	100
	CBA HC_{KLH}	TNP-KLH	B10.HTT SF_{KLH}	–	160 ± 38	35
	CBA HC_{KLH}	TNP-KLH	B10.HTT SF_{KLH}	CBA spleens 10^8/ml	110 ± 36	22
	CBA HC_{KLH}	TNP-KLH	B10.HTT SF_{KLH}	CBA ATX + ALS spleens 10^8/ml	157 ± 28	35
	CBA HC_{KLH}	TNP-KLH	B10.HTT SF_{KLH}	CBA spleens T 0.5 × 10^8/ml	187 ± 57	43
II	–	TNP-KLH	–	–	627 ± 42	0
	CBA HC_{KLH}	TNP-KLH	–	–	1,423 ± 99	100
	CBA HC_{KLH}	TNP-KLH	CBA SF_{KLH}	–	663 ± 55	5
	CBA HC_{KLH}	TNP-KLH	CBA SF_{KLH}	CBA MPh monolayer	423 ± 33	0
III	–	TNP-KLH	–	–	37 ± 13	0
	CBA HC_{KLH}	TNP-KLH	–	–	450 ± 56	100
	CBA HC_{KLH}	TNP-KLH	C57Bl/10 SF_{KLH}	–	63 ± 32	6
	CBA HC_{KLH}	TNP-KLH	C57Bl/10 SF_{KLH}	CBA HC_{KLH} 3 × 10^6/ml	476 ± 79	100
	CBA HC_{KLH}	TNP-KLH	C57Bl/10 SF_{KLH}	C57Bl HC_{KLH}	620 ± 150	100
	CBA HC_{KLH}	TNP-KLH	C57Bl/10 SF_{KLH}	C57Bl HC (T, G)-A--L	413 ± 118	91
IV	–	TNP-KLH	–	–	283 ± 68	0
	CBA HC_{KLH}	TNP-KLH	–	–	800 ± 107	100
	CBA HC_{KLH}	TNP-KLH	CBA SF_{KLH}	–	243 ± 27	0
	CBA HC_{KLH}	TNP-KLH	CBA SF_{KLH}	CBA SC_{KLH} 10^7/ml	13 ± 3	0

SF_{KLH} at a final concentration of 10% of CBA, B10.HTT or C57BL/10 origin either untreated or absorbed with normal spleen cells, ATX + ALS spleens, splenic T cells, macrophages (MpH) or in vitro-induced HC or SC was added to the mixture of CBA in vitro-primed HC_{KLH} and normal CBA spleen cells. The mixture was challenged with TNP-KLH and the numbers of anti-DNP antibody-forming cells assayed after 4 days culture in vitro. Two to five experiments of each type gave analogous results.

our interest in induction of various cooperative pathways, we have developed an in vitro model of antigen-specific SC induction (5, 21) and we have used these in vitro-induced SC to analyze regulatory pathways, both in vitro and in vivo (unpublished observations). We describe in this communication the mechanism of action of these SC via a secreted SF, which acts in a manner quite distinct from the extracts of SE. These differences are striking, and they emphasize the heterogeneity of suppressive interactions and the complexity of the regulation of the immune system.

Although it is very difficult to prove unequivocally that any factor involved in cell cooperation works physiologically better at a distance from the cell which produces it, there is evidence to suggest that in vitro SC do function by releasing SF. First, only metabolically active SC release SF. Second, such small numbers of SC are sufficient to suppress in vitro primary responses, e.g. 10^{3-4}, that direct contact is difficult to envisage. It was thus of interest to ascertain the target of SF action. Theoretically, this could be at any level before the secretion of antibodies by B cells, although prior evidence of the carrier specificity of SF action and the lack of effect on thymus-independent responses (5, 21) implied a target somewhere in the helper pathway. A simple experiment to focus on the target of SF is to compare its effect on cultures stimulated by HC or HF. This would localize the effect of SF before or after the release of HF. The results in Table III and other experiments by Howie et al. (unpublished observations) clearly indicate that once HF has been released, the SF is inert. The target of SF is the helper pathway (the HC or some

TABLE VIII
Lack of Genetic Restriction in the Action of SF

Stimulus		Suppression	Response			
			Anti-DNP-AFC/culture ± standard error			
HC	Antigen		IgM		IgG	
				%		%
CBA TNP-KLH-primed	–	–	1,040 ± 323	0	0	0
	TNP-KLH	–	6,560 ± 611	100	19,493 ± 2,218	100
	TNP-KLH	CBA SF_{KLH} 30%	2,560 ± 167	28	6,400 ± 400	33
	TNP-KLH	CBA SF_{KLH} 10%	3,173 ± 615	39	4,000 ± 1,533	20
	TNP-KLH	B10.Br SF_{KLH} 30%	6,000 ± 500	90	12,933 ± 2,779	66
	TNP-KLH	B10.Br SF_{KLH} 10%	4,480 ± 212	62	6,080 ± 1,257	31
	TNP-KLH	SJL SF_{KLH} 30%	4,133 ± 938	56	6,960 ± 642	36
	TNP-KLH	SJL SF_{KLH} 10%	3,504 ± 1,379	45	13,680 ± 2,598	70
(CBA × B10)F_1 TNP-KLH-primed	–	–	60 ± 23	0	7 ± 7	0
	TNP-KLH	–	4,000 ± 381	100	9,483 ± 100	100
	TNP-KLH	CBA SF_{KLH} 30%	1,633 ± 501	40	4,116 ± 996	43
	TNP-KLH	B10 SF_{KLH} 30%	1,900 ± 480	47	3,017 ± 697	32
	TNP-KLH	B10.HTT SF_{KLH} 30%	2,567 ± 1,288	64	3,583 ± 211	38
	TNP-KLH	SJL SF_{KLH} 30%	2,550 ± 775	63	5,717 ± 2,685	60
	TNP-KLH	B10.HTT SF_{GAT} 30%	7,433 ± 543	>100	16,883 ± 1,831	>100

Secondary in vitro responses by TNP-KLH-primed CBA or (CBA × B10)F_1 spleen cells challenged with TNP-KLH in vitro in the absence or presence of SF_{KLH} at a final concentration of 10 or 30% of CBA, B10.Br, B10. B10.HTT or SJL origin. The numbers of anti-DNP antibody-forming cells were assayed on day 6 of culture. Two other experiments gave analogous results.

TABLE IX
Summary of Strains of Origin of SF_{KLH} Tested in Primary Responses in Vitro

Origin of SF_{KLH}		Suppression		I-Region similarities between SF/HC	
H-2 Haplotype	Strain	CBA HC_{KLH}	B10.HC_{KLH}	CBA	B10
k	CBA, B10.Br	Yes	Yes	All	None
b	C57Bl, B10	Yes	NT*	None	NT
a	B10.A, A/Sn, A/J	Yes	NT*	A,B,J,E	NT
h4	B10.A (4R)	Yes	NT*	A,	NT
i5	B10.A (5R)	Yes	NT*	J,E	NT
t3	B10.HTT	Yes	Yes	E,C	None
s	SJL	Yes	NT	None	None

* NT, not tested.

accessory cell, before the release of HF) and clearly not the B cell or the macrophage which interacts with it and HF. Experiments to define the target more precisely were performed by purifying the cells in the helper cell pool.

The target of SE described by Tada et al. (37, 41) was a primed, nylon wool-adherent T cell with the Ly-$1^+2^+3^+$ I-J^+ membrane phenotype, which is not a helper cell. Thus, we ascertained the membrane phenotype of the suppressible cell within the helper cell pool. Table IV indicates that it was not adherent to nylon wool. Table V indicates that it was an Ly-1^+ cell, as treatment with anti Ly-2.1 serum and complement, which would kill both Ly-$1^+2^+3^+$ cells and Ly-2^+3^+ cells had no effect on the capacity of SF to inhibit the in vitro response.

Our results were different from those of Tada: the suppressible cell here is $Ly1^+$, and nylon wool nonadherent. However, since our assay system also

TABLE X
A Comparison of Antigen-Specific Suppressive Products

	Suppressor factor Kontiainen and Feldmann	Suppressor extracts	
		Tada et al. (24-26)	Benacerraf/Kapp et al. (27-29)
Cellular source	Thy-1$^+$ Ly1$^-$2$^+$3$^+$Ia$^+$ antigen-specific suppressor cells	Thy-1$^+$ Ly1$^-$2$^+$3$^+$ (I-J) antigen-specific suppressor cells	
Produced by	Metabolically active cells after further in vitro culture	Sonication extracts of suppressor cells	
Producer strains	All tested (see text)	All but A/J	Nonresponder strains
Suppressible stains	CBA, B10	B10 and B10 congenics not suppressible	Mostly nonresponder strains
Target cells within helper-cell population	Nylon wool-nonadherent Ly2$^-$	Nylon wool-adherent Ly1$^+$2$^+$3$^+$Ia$^+$ (I-J)	T cell (?)
Genetic restriction	No	I-J syngeneity required	Not always demonstrable
Possible mechanism of action	Inactivation of HC	Recruitment of more suppressor cells	

contains unprimed T cells, it was possible that these (or perhaps an Ly-1$^+$2$^+$3$^+$ cell among them) were involved in some interaction with SF. Experiments were performed using HC treated with anti-Ly2 and C′ (excluding primed Ly1$^+$2$^+$3$^+$ cells) and anti-θ- and C′-treated spleen cells as a source of B cells, thus depleting unprimed Ly1$^+$2$^+$3$^+$ cells or any other unprimed T cells. Again, SF was just as effective in the absence of these cells as in their presence (Table VI). The above experiments indicate that the target of in vitro SF was an Ly-1$^+$ nylon wool nonadherent cell within the HC pool. This is of the same phenotype as the helper cell, but it is not certain that the target is the HC. Theoretically, it may be another cell of the same membrane phenotype which then transmits its signal to the antigen-specific helper cell itself. Further experiments are necessary to distinguish between these possibilities.

Genetic restrictions of cell interaction have been reported in several circumstances; in T/B-cell interactions (42), in T-macrophage interaction (43, 44), in mixed leucocyte cultures between SC and mixed leucocyte-reactive cells (45), and also in suppressor cell-target cell interactions (26). In the latter circumstances, the genetic restrictions were mapped to *I-J* region (26). Analogous experiments were thus performed with SF, SF_{KLH}, $SF_{(T,G)\text{-}A\text{-}L}$, and SF_{GAT} on several strains. No evidence for genetic restrictions was obtained, even with limiting dilution of SF, in either primary (Tables IV, V, IX) or secondary responses (Table VIII). The results shown here are only with SF_{KLH}, those with $SF_{(T,G)\text{-}A\text{-}L}$ or SF_{GAT} will be detailed elsewhere. It should be pointed out that not all SE are genetically restricted and require homology at the *I-J* region. Kapp et al. (28), using suppressor extracts of cells primed to GAT, found that these worked on allogenic strains, but only if they were GAT nonresponders. SE to a copolymer of L-glutamine-L-tyrosine appears to work in allogeneic or syngeneic nonresponder strains (46).

The discussion so far has made it clear that contrasting results have been obtained with SF and SE. For easy comparison these have been tabulated (Table X), and they extend beyond the methodological and suggest strongly that SF and SE are not the same molecules. Other differences not discussed in detail here are among the strains of mice which produce SF or SE and which are suppressible. Further differences include experiments with A/J mice which

do not make SE, but make SF in vitro, and B10 and B10 congenic mice which are not suppressible by SE, but are inhibited by in vitro induced SC (22) or SF. A question of interest concerns the respective roles of these two molecules. This is a topic which has attracted much speculation. There is evidence that SE may be important for the recruitment of SC (37), and it is possible that this is its major function. Recently, Tada and his colleagues have proposed a scheme whereby $Ly2^+$ 3^+ cells shed $I\text{-}J^+$ SE, which acts on primed $Ly1^+2^+3^+$ $I\text{-}J^+$ cells, which then differentiate to become SC, with the SE being a component of a SC recruitment loop. More recent results, however, suggest that $Ly1^+2^+3^+$ cells interact with $Ly2^+3^+$ cells but do not themselves differentiate into $Ly2^+3^+$ cells (Tada, personal communication). On the basis of the data reported here, we think that SF may act at a different locus by inactivating HC, indicating that it is much more of an effector molecule than SE. This still leaves the relationship of SE to SF unresolved. Are these totally unrelated or part of a family of suppressive molecules, or do they have a precurser relationship? Our studies to biochemically characterize SF are still in their infancy, but there is evidence that SF, like SC, is $I\text{-}J^+$, indicating a family relationship. This may be clarified further by the use of heterologous antisera to SF which recognizes a constant region on all SF molecules.[2]

Summary

Antigen-specific suppressor factor produced by metabolically active in vitro-induced suppressor cells, upon further antigenic stimulation, act on nylon wool nonadherent, Ly-2-negative target cells within helper cell population, resulting in suppression of both the IgM and IgG antibody responses. Thus the target is an $Ly\text{-}1^+$ T cell, possibly the helper cell. All the mouse strains tested so far have been able to produce the factor, and when tested in CBA or B10 mice, there seems to be no genetic restriction involved e.g., nonsyngeneic suppressor factors suppress as well as do the syngeneic factors. Comparison of the properties of suppressor factor with those of extracts of suppressor cells yield differences in origin, target of action and effect, indicating that these are different molecules. The heterogeneity of suppressor pathways is discussed.

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