INTERMEDIARY ROLE OF MACROPHAGES IN THE PASSAGE OF SUPPRESSOR SIGNALS BETWEEN T-CELL SUBSETS*

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Mice that are injected with picryl sulphonic acid (PSA; 2,4,6-trinitrobenzene sulfonic acid)[1] before they are sensitized with picryl chloride (PCL), develop Thy-1$^+$ suppressor cells which can abrogate the ability of immunized T cells to transfer skin-contact hypersensitivity (1). These Thy-1$^+$ suppressor cells have been shown to release a factor(s) (SF) which, when incubated with immune T cells in vitro, prevents the T cells from conferring adoptive immunity (2). The SF has antigen specificity, since it only inhibits the adoptive transfer of contact hypersensitivity to PCL (when PCL is used to produce the factor) and not the adoptive transfer of immunity to other skin-sensitizing reagents. The SF can be adsorbed from culture supernates by Thy-1$^-$ peritoneal exudate cells (PEC) (2).

We have tested the hypothesis that the interaction of the SF with these PEC, which exhibit multiple macrophage (Mϕ)-like qualities and are thus hereafter referred to as Mϕ, is an important if not obligatory step in the specific suppression of previously immunized cells. Towards that end, we have shown that Mϕ, both live (L Mϕ) and dead (HK Mϕ), can adsorb the SF from culture supernates with approximately the same efficiency. When immunized lymphocytes are incubated with L Mϕ that have been used to adsorb SF, the immunized lymphocytes lose the ability to transfer immunity. However, when immunized lymphocytes are mixed with HK Mϕ that have been used to adsorb SF, no suppression is seen. The inability of the HK Mϕ to successfully present suppressor activity to T cells is not due to inactivation of the SF, since it can be eluted from the HK Mϕ by normal L Mϕ. The L Mϕ used for elution attain specific suppressor activity which is equivalent to that of L Mϕ incubated

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[1] *Abbreviations used in this paper:* CS, control supernate; HAGG, heat-aggregated gamma globulin; HK Mϕ, heat-killed (dead) macrophages; i.p., intraperitoneally; i.v., intravenously; L Mϕ, live macrophages; MEM, minimal essential medium; MHC, major histocompatibility complex; Mϕ, macrophage(s); PCL, picryl chloride; PEC, peritoneal exudate cells; PSA, picryl sulphonic acid (2,4,6-trinitrobenzene sulphonic acid); SAF, suppressor arming factor; SF, suppressor factor; SIRS, nonspecific suppressor factor.

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directly in SF. The ability of HK Mϕ to adsorb suppressor activity can be blocked by prior incubation of the Mϕ with heat-aggregated immunoglobulin.

Thus, these results, taken in context with previous studies (2–5), show that one type of suppressive interaction between T-cell subsets occurs via a Mϕ intermediary; that the receptor on the Mϕ membrane that is used for specific arming has some characteristics that are similar to Fc receptors; and that the suppressor signal delivered by the Mϕ requires some biological activity on the part of the Mϕ—most likely the production of a factor which effects the suppression. The role of the T-cell product may be simply to serve as a passively acquired antigen-specific receptor for Mϕ, or perhaps for a specific (i.e., suppressor) Mϕ subpopulation.

Materials and Methods

Animals. 8–10-wk-old CBA mice of the Harwell substrain, obtained from either the Jackson Laboratory, Bar Harbor, Maine, or from the animal colony of the Copernicus Medical Academy, Cracow, Poland, were used in transfer experiments. In some experiments, C57BL/6, DBA2, or closed-colony-bred Swiss mice from the Copernicus Medical Academy were also used. Outbred guinea pigs were purchased from a local supplier in Poland.

PEC. Mice were injected intraperitoneally (i.p.) with 2.5 ml of thioglycollate medium (Difco Laboratories, Detroit, Mich.), and PEC were collected 4 days later. Peritoneal exudates were induced in guinea pigs by an i.p. injection of 25 ml of liquid paraffin. Harvested PEC were killed by heating in a 56°C water bath for 45 min as described previously (3). These cells will be referred to hereafter as Mϕ, because purification studies have shown that after heat-killing, only the Mϕ fraction of PEC retains the activities we have studied (3).

Tumor Cell Lines. L1210 and P815 were propagated by serial passage in the peritoneal cavities of DBA2 mice.

Sensitization Procedure. Mice were sensitized by the application of 0.15 ml of 7% PCL (Fluka AG, Basel, Switzerland) or 3% oxazolone (British Drug Houses, Ltd., Poole, England) in absolute ethanol to the skin of the clipped abdomen and all four paws.

Adoptive Transfer Experiments. 4 days after sensitization with PCL, or 5 days after sensitization with oxazolone, CBA mice were killed and their spleens and peripheral lymph nodes were removed. Cell suspensions were prepared by gentle teasing. The resultant cell suspensions were injected intravenously (i.v.) into syngeneic recipients in a 0.3-ml volume. Each recipient mouse received 4×10^7 cells. Immediately after cell transfer, the recipients were challenged by application of 1% PCL or oxazolone in olive oil to both sides of the ear. Ear swelling was measured in micrometer units (1 U = 0.01 cm) after 24 and 48 h (6). Since no differences were found between 24- and 48-h reactions, only the former results are presented below (± SD). Each experimental group consisted of 4–6 animals.

Preparation of SF and Control Supernates (CS). SF was prepared as described by Zembala and Asherson (2) with minor modifications. CBA mice were injected i.v. twice; once on day 0 and again 3 days later with 0.35 ml of PSA (1% solution). On day 6 the mice were skin painted with 7% PCL, and 18 h later single cell suspensions were prepared from the spleen and peripheral lymph node cells. The suspensions were incubated in minimal essential medium (MEM) supplemented with glutamine, pen-strep mixture, and 2% fetal calf serum at a cell density of $1.5–2 \times 10^7$ cells/ml. Supernates were harvested 48 h after the start of culture, and these supernates are hereafter referred to as SF. CS were prepared by a similar incubation of cells from mice that had not been injected with PSA before skin painting with PCL, or that were only injected with PSA without painting.

Heat-Aggregated Mouse Gamma Globulin. Mouse gamma globulin was prepared by treatment of serum with 33% of ammonium sulfate. After extensive dialysis against saline, the concentration of the precipitated protein was adjusted to 10 mg/ml. Preparations were heat-aggregated at 62°C for 1 h, and they were used within 24 h of treatment. HK Mϕ were incubated with these preparations for 1 h at 37°C using 1 ml/2×10^7 cells.

Preparation of Nylon Wool Column. Nylon wool was obtained from Fenwall Laboratories, Deerfield, Ill. and prepared as described by Julius et al. (7). In those experiments where the nylon wool was used to remove HK Mϕ from lymphocytes, the elution of the cells from the columns was done very slowly, as the HK Mϕ did not adhere very strongly to the nylon. However, using the slow elution, <1% of the HK Mϕ passed through the nylon wool.

Experimental Protocol Used in "Adsorption" Experiments. Cells from immunized mice were incubated in either CS or SF, at a concentration of 4×10^7 cells/ml for 1 h at 37°C. After this they were washed once and injected i.v. into recipients. In some experiments, CS or SF were adsorbed before use with HK Mϕ or heat-killed L1210 or P815 tumor cell lines. Adsorption was done for 1 h at 37°C using 2×10^7 HK cells/ml unless otherwise stated.

Experimental Protocol Used in "Arming" Experiments. Syngeneic (CBA) or allogeneic (C57BL/6) Mϕ were incubated in SF or CS. 1×10^7 cells/ml were incubated for 1 h at 37°C. The Mϕ used for adsorption were washed and then mixed with immune spleen and lymph node cells at a ratio of 1×10^7 L Mϕ or 2×10^7 HK Mϕ to 5×10^7 sensitized lymphocytes for 1 h at 37°C in 1 ml of MEM supplemented with 3% fetal calf serum. After incubation, cells were separated on nylon wool columns (see separation technique above), and the effluent cells were injected into recipients i.v. In these experiments, 2×10^7 sensitized T cells were injected into each recipient mouse. In addition, a positive control was performed; this consisted of transference of T cells that had been incubated in CS without any Mϕ and then column-purified, as described above.

Results

Adsorption Studies

ABILITY OF HK Mϕ TO ADSORB SF. As shown in Table I, immune lymphocytes incubated in CS produced a significant 24-h ear swelling reaction (compare groups 1 and 5). When the immunized cells were incubated in SF, their ability to transfer adoptive immunity was significantly suppressed (group 2). All activity was removed from SF by preincubation with suspensions of L (group 3) or HK (group 4) Mϕ at a concentration of 2.7×10^7/ml. Lower doses of HK Mϕ were unable to adsorb significant SF activity.

SPECIFICITY OF ADSORPTION OF SF BY HK Mϕ. As seen in Table II, HK Mϕ from syngeneic CBA mice, allogeneic Swiss mice, or xenogeneic guinea pigs (groups 3–5) were able to adsorb all the suppressor activity demonstrated by the decreased reactivity of mice in group 2 (compare group 1). On the other hand, other cells with Fc receptors, such as (heat-killed) L1210 and P815 tumor line cells, failed to adsorb SF activity (groups 6 and 7).

BLOCKING ADSORPTIVE CAPACITY OF HK Mϕ WITH AGGREGATED Ig. The ability of HK Mϕ to adsorb SF, (group 3, Table III) was lost when the HK Mϕ were pretreated with heat-aggregated gamma globulin (HAGG) (group 4). The presence of HAGG on the HK Mϕ did not alter the immune capacity of the lymphocytes that had not been incubated in SF (compare groups 5 and 6), indicating that the HAGG carried on HK Mϕ did not directly affect the immune lymphocytes.

Arming Mϕ with SF. The above experiments show that Mϕ can adsorb SF activity. To determine the biological significance of this adsorption, we next did a series of experiments to determine if the SF that had been adsorbed by the Mϕ retained biological activity. Thus, we asked the Mϕ that had been used for adsorption to present SF to the immunized lymphocytes.

Neither HK Mϕ nor L Mϕ that had not been incubated in SF affected the adoptive transfer of immunity when incubated with the immune cells and subsequently removed from them on nylon columns (Table IV, groups 2 and 3).

TABLE I
Ability of HK Mϕ to Adsorb SF

Group	Treatment of sensitized cells with:	Ear swelling ($\times 10^{-3}$ cm)
1	CS (positive control)	8.6 ± 1.56
2	SF	3.2 ± 1.11
3	SF adsorbed with 2.7×10^7/L Mϕ	8.8 ± 1.72
4	SF adsorbed with (x) HK Mϕ:	
	$x = 2.7 \times 10^7$/ml	8.5 ± 1.90
	$x = 0.9 \times 10^7$/ml	6.6 ± 1.31
	$x = 0.3 \times 10^7$/ml	3.7 ± 1.29
5	No transfer (negative control)	1.3 ± 0.52

4×10^7 sensitized lymphocytes were incubated for 1 h at 37°C in either CS (group 1) or SF (group 2), washed once, and injected into nonimmune recipients. In some experiments, SF was adsorbed before being used with L Mϕ (group 3) or HK Mϕ (group 4) for 1 h at 37°C. (Adsorption of control supernate with Mϕ did not change its properties.)

TABLE II
Specificity of Adsorption of SF by HK Mϕ

Group	Treatment of sensitized cells with:	Ear swelling ($\times 10^{-3}$ cm)
1	CS	7.6 ± 1.74
2	SF	3.9 ± 1.31
3	SF adsorbed CBA HK Mϕ	6.9 ± 1.52
4	SF adsorbed Swiss HK Mϕ	8.6 ± 1.76
5	SF adsorbed guinea pig HK Mϕ	7.8 ± 1.22
6	SF adsorbed DBA/2 HK L1210	3.7 ± 1.41
7	SF adsorbed DBA/2 HK P815	2.8 ± 1.44
8	No transfer (negative control)	1.2 ± 0.40

4×10^7 sensitized lymphocytes were incubated for 1 h at 37°C in either CS (group 1) or SF (group 2), washed once, and injected into nonimmune recipients. In some experiments, SF was adsorbed before being used with different cell types (groups 3–7).

TABLE III
Inhibition of the Ability of HK Mϕ to Adsorb SF by HAGG

Group	Treatment of sensitized cells with:	Ear swelling ($\times 10^{-3}$ cm)
1	CS	9.8 ± 1.20
2	SF	4.5 ± 1.56
3	SF adsorbed HK Mϕ	8.3 ± 1.00
4	SF adsorbed HK Mϕ-HAGG	4.6 ± 0.97
5	CS adsorbed HK Mϕ	8.9 ± 1.31
6	CS adsorbed HK Mϕ-HAGG	10.3 ± 0.99
7	No transfer (negative control)	1.9 ± 0.60

SF or CS were adsorbed before incubation with sensitized cells with HK Mϕ or HK Mϕ that had been treated previously with HAGG.

TABLE IV
Ability of Syngeneic Mϕ to Actively Present SF to Immune Lymphocytes

Group	Incubation of sensitized cells with:	Ear swelling ($\times 10^{-3}$ cm)
1	Medium only (positive control)	4.5 ± 0.62
2	HK Mϕ	4.1 ± 0.71
3	L Mϕ	4.3 ± 0.55
4	HK Mϕ-SF	4.6 ± 0.82
5	L Mϕ-SF	1.3 ± 0.57
6	HK Mϕ-SF + L Mϕ (groups 3 & 4)	1.0 ± 0.20
7	No transfer (negative control)	0.4 ± 0.20

Sensitized lymphocytes were incubated for 1 h at 37°C with various syngeneic Mϕ preparations. The Mϕ were then removed on nylon columns and the effluent lymphocytes were injected into nonimmune recipients.

HK Mϕ that had been used to adsorb SF also did not affect adoptive transfer of immunity (group 4). On the other hand, L Mϕ incubated in SF were highly suppressive (group 5). The inability of the HK Mϕ, which had been used to adsorb SF, to inhibit the adoptive transfer of immunity was not due to inactivation of the SF by the HK Mϕ, since the addition of untreated L Mϕ to these cells resulted in a significant suppression (group 6), indicating that the L Mϕ were able to retrieve the SF in active form from the surfaces of the HK Mϕ.

Lack of Requirement for H-2 Compatibility for Mϕ Presentation of SF to Immunized Lymphocytes. Since we knew that there was no H-2 requirement for Mϕ adsorption of SF, we asked whether any restrictions could be found in the Mϕ presentation of SF. The results in Table V confirm that L Mϕ not incubated in SF have no suppressive activity and that HK Mϕ have no suppressive activity; they also show that macrophages that are allogeneic to the cells which produce SF as well as to the target cells, can present SF very efficiently. The results in Table VI are from another experiment. These confirm the three points made above: (*a*) HK Mϕ that had been used to adsorb SF cannot actively present that factor to immunized lymphocytes (groups 4 and 5); (*b*) the factor is not inactivated because L Mϕ can retrieve it in an active form from the HK Mϕ (groups 6 and 7); and (*c*) there is no identity required at the H-2 locus for the active presentation of the SF by the L Mϕ (group 7).

Lack of Requirement for Lymphocytes as Intermediaries in the Retrieval of SF from HK Mϕ by L Mϕ. In the previous experiments, we showed that the addition of L Mϕ to HK Mϕ that had been used to adsorb SF resulted in a return of suppressor activity when immune lymphocytes were added to the Mϕ mixtures. To determine whether the added lymphocytes played any role in helping to retrieve suppressor activity from the HK Mϕ, we performed an experiment in which the HK Mϕ and the L Mϕ were mixed in the absence of added lymphocytes, and the HK Mϕ were subsequently removed from culture. We then asked whether the L Mϕ could actively present suppressor activity in the absence of the HK Mϕ that had been the carrier, so to speak, of SF. The protocol we used to achieve these results is as follows. 5×10^6 PEC were suspended in MEM and 2% fetal calf serum. The Mϕ were then plated on Falcon 3001 dishes (Falcon Products, Div. Becton Dickinson & Co., Cockeysville, Md.) for 2 h at 37°C. Nonadherent cells were removed and the Mϕ monolayers were

TABLE V
Lack of Requirement for H-2 Compatibility for Mϕ Presentation of SF to Immunized Lymphocytes

Group	Incubation of sensitized cells with:	Ear swelling ($\times 10^{-3}$ cm)
1	Medium only (positive control)	4.6 ± 0.87
2	HK Mϕ	4.6 ± 0.91
3	L Mϕ	4.8 ± 0.57
4	HK Mϕ-SF	5.2 ± 0.64
5	L Mϕ-SF	1.7 ± 0.73
6	No transfer (negative control)	0.9 ± 0.27

Sensitized lymphocytes (CBA) were incubated for 1 h at 37°C with various allogeneic (C57BL/6) Mϕ preparations. The Mϕ were then removed on nylon columns and the effluent lymphocytes were injected into nonimmune CBA recipients.

TABLE VI
Ability of L Mϕ to Retrieve SF from HK Mϕ

Group	Incubation of sensitized cells with:	Ear swelling ($\times 10^{-3}$ cm)
1	Medium only (positive control)	5.4 ± 1.09
2	L $M\phi_{CBA}$	4.8 ± 0.94
3	L $M\phi_{C57}$	4.8 ± 0.57
4	HK $M\phi_{CBA}$-SF	5.1 ± 0.63
5	HK $M\phi_{C57}$-SF	5.2 ± 0.64
6	HK $M\phi_{CBA}$-SF + L $M\phi_{CBA}$	1.8 ± 0.43
7	HK $M\phi_{C57}$-SF + L $M\phi_{C57}$	2.2 ± 0.47
8	No transfer (negative control)	0.8 ± 0.10

Sensitized CBA lymphocytes were incubated for 1 h at 37°C with various syngeneic (CBA) or allogeneic (C57BL/6) Mϕ preparations. The Mϕ were then removed on nylon columns and the effluent lymphocytes were injected into nonimmune CBA recipients.

exposed to either 1 ml of SF or 2×10^7 HK Mϕ that had been previously incubated in SF or CS. After 1 h of incubation at 37°C, the HK Mϕ (and any nonbound SF) were removed by vigorous rinsing. After rinsing, <0.5% of the added HK Mϕ was found adhering to the L Mϕ (i.e., >99.5% of the cells remaining excluded trypan blue). After this rinsing procedure, 5×10^7 sensitized lymphocytes were added to the Mϕ monolayers for an additional 1 h at 37°C. After this time, the immunized lymphocytes were washed off the monolayers and transferred into nonimmune recipients. The results in Table VII show that the lymphocyte incubated on the Mϕ monolayers that had never seen SF transferred good adoptive immunity (groups 1 and 3). On the other hand, lymphocytes incubated with Mϕ monolayers that had SF applied directly (group 2), or applied indirectly via the HK Mϕ that had been incubated in SF (group 4) were significantly inhibited in their ability to transfer adoptive immunity. It thus appears that L Mϕ can retrieve SF from HK Mϕ without the requirement for a lymphocyte intermediary cell.

Specificity of the Suppression Produced by L Mϕ Armed with SF. The results in Table VIII show that both syngeneic (group 2) and allogeneic (group 3) L Mϕ that have retrieved SF from HK Mϕ markedly suppress the adoptive transfer of immunity to PCL (the antigen used for producing SF), but do not

TABLE VII
Ability of L Mϕ to Retrieve SF from HK Mϕ in the Absence of Lymphocytes

Group	Incubation of sensitized lymphocytes on L Mϕ monolayers pretreated with:	Ear swelling ($\times 10^{-3}$ cm)
1	CS (positive control)	7.0 ± 0.79
2	SF	1.4 ± 0.51
3	HK Mϕ	6.6 ± 0.82
4	HK Mϕ + SF	3.2 ± 0.71
5	No transfer (negative control)	1.2 ± 0.65

Mϕ monolayers (L Mϕ) were incubated with medium (group 1) or SF (group 2) or HK Mϕ (group 3) or HK Mϕ that had been pretreated with SF (group 4). After vigorous washing, sensitized lymphocytes were added to the monolayers for 1 h at 37°C, and then removed by washing, and injected into nonimmune recipients.

TABLE VIII
Specificity of Suppression Produced by L Mϕ Armed by SF

Group	Incubation of sensitized lymphocytes with:	Reagent used to sensitize lymphocytes	
		Ear swelling ($\times 10^{-3}$ cm) PCL	Oxazolone
1	Medium only (positive transfer)	4.2 ± 0.40	5.5 ± 0.43
2	HK $M\phi_{CBA}$-SF (PCL) + L $M\phi_{CBA}$	1.0 ± 0.14	4.6 ± 0.93
3	HK $M\phi_{C57}$-SF (PCL) + L $M\phi_{C57}$	1.5 ± 0.14	5.3 ± 0.94
4	No transfer (negative control)	0.9 ± 0.20	1.9 ± 0.32

Sensitized lymphocytes were incubated for 1 h at 37°C with various syngeneic Mϕ preparations. The Mϕ were then removed on nylon columns and effluent lymphocytes were injected into nonimmune recipients.

effect the adoptive transfer of immunity to oxazolone. As in previous experiments (except those in which monolayers were used), the lymphocytes were purified by nylon column fractionation after incubation with the Mϕ. It should be stressed that although no exogenous antigen was added to the incubation mixtures of lymphocytes and Mϕ, there was almost certainly some antigen present on the sensitized cells, and it is most likely that this antigen was involved in triggering the armed Mϕ to produce its suppressor effects. Thus, these experiments attest to the specificity of the SF, and not to the specificity of the suppression produced by the triggered Mϕ.

Discussion

The results we have presented establish the following points.

(*a*) Peritoneal exudate Mϕ membranes have structures which can adsorb a previously described SF (2), and the adsorbing capacity of the Mϕ is passive, since HK Mϕ can adsorb activity as well as L Mϕ.

(*b*) There is no requirement for histocompatibility or even species compatibility between the Mϕ and the SF for adsorption to take place.

(*c*) The ability of the Mϕ cell surface structure to adsorb the suppressor factor can be blocked with heat-aggregated immunoglobulin. This observation and other studies showing correlation between the ability of HK Mϕ to block

communication between T cells and the presence of a demonstrable Fc receptor (5) suggest that the receptor on the Mϕ for the SF has Fc-like characteristics. It is important to note that this does not mean it is a classical Fc receptor. In fact, there are a number of reasons to think that it is not, the most compelling of which is the inability of some cells with known Fc to adsorb SF (see Table II). The concept of subtypes of Fc receptors is now being recognized (8, 9), and it would seem most likely that the cell surface structure on the Mϕ that we have described will eventually turn out to be one of these subtypes.

(*d*) Although HK Mϕ can adsorb SF, they cannot actively present it in a fashion which inhibits T-cell activity; only L Mϕ can perform the latter function. Since the SF we are studying works by giving Mϕ passive antigen specificity, it would be more accurate to call it suppressor arming factor (SAF). This does not necessarily mean that we know the Mϕ is an obligatory intermediary in the transfer of suppression from effector to target T cells, or that other factors that may not work with a Mϕ as an intermediary cell are not also made. It simply emphasizes that the specific T-cell factor we have described works by creating a specific suppressor Mϕ, and that we have no evidence for any other SF, since HK Mϕ adsorb all suppressor activity. In fact, all known suppressor factors may be completely adsorbable by Mϕ and/or require the biological activity of a Mϕ to function.

(*e*) The inability of HK Mϕ to present SF in a functional way is not due to inactivation of the SF, since specific suppressor activity can be transferred from nonfunctional HK Mϕ to L Mϕ. The mechanism by which SF is transferred from the HK Mϕ to the L Mϕ is of some interest. Since HK Mϕ do not release SF into culture supernates after they have adsorbed it, it would seem that an energy-requiring activity on the part of the L Mϕ is necessary for removing the factor from the HK Mϕ. It is possible that the energy requirement is for stabilization of the bond between the SAF and the membrane receptor so that the balance of an on-off reaction is switched in favor of the L Mϕ. Alternatively, the transfer from HK to L Mϕ may reflect some form of Mϕ:Mϕ interaction which could serve as a focusing device to help concentrate T-cell communication signals on appropriate Mϕ subpopulations. The ability to define functionally distinct Mϕ subpopulations with anti Ia sera (10) should help to resolve this potentially important question.

The notion that the transference of SAF from a Mϕ to another cell is not some peculiar artifact created in our test tubes by using HK Mϕ (which are obviously not important physiological entities) is suggested by some previous work. L Mϕ that have been incubated with SAF and then inoculated i.v. along with sensitized lymphocytes into nonimmunized mice can specifically inhibit the contact hypersensitivity response of the recipient mice (2). This result was initially interpreted to indicate that the injected Mϕ migrated to the site where the contact hypersensitivity response was taking place and effected their immunosuppression at that site (11). This explanation seems implausable to us since adoptively transferred peritoneal exudate Mϕ do not migrate in significant numbers anywhere, except perhaps to the lungs and liver (12). It seems more likely that some type of receptor transfer between the inoculated armed Mϕ and some other host cells is required for the transportation of the suppressor signal

to its site of action. This latter interpretation is also supported by our findings that HK Mϕ that have been used to adsorb suppressor activity and that are unable to function as armed suppressor cells in vitro can suppress the immune response of sensitized mice just as efficiently as L Mϕ if they are injected in vivo.[2]

Our results also indicate that lymphocytes are not obligatory intermediaries in the transfer of suppressor information from the HK Mϕ to the L Mϕ, since the transfer can be accomplished in vitro in the absence of significant numbers of lymphocytes. This observation, as well as a total lack of histocompatibility requirements for effective transfer of the information between either the cell which makes SAF and the Mϕ which it arms, or between the target T cells and the armed Mϕ, distinguishes the factor we have described from genetically restricted factor described by Erb and Feldmann (13). On the other hand, there is some similarity between our findings and the earlier work of Feldmann and Nossal (14) which suggested an important intermediary role for the Mϕ in the transmission of T-cell derived signals. In addition, the work of Tadakuma and Pierce (15) suggesting that the Mϕ is the target cell for the nonspecific suppressor factor (SIRS) induced from Ly-23 cells by concanavalin A is probably also related to our findings. It is possible that the SIRS actually contains specific SAF which is made as a result of polyclonal, rather than nonspecific, activation.

Previous studies have suggested that a factor made in exactly the same fashion as our factor may contain determinants coded for in the major histocompatibility complex (MHC) (16). However, it is not certain that these determinants are in SAF or in some other factor present in the same culture supernates. Studies on the role of MHC-coded determinants on SAF and its producer cell, as well as on its Mϕ targets cell, are presently underway.

The question as to the mechanism by which the specifically armed Mϕ produces the suppression of immune T cells remains. Two major possibilities suggest themselves. (*a*) The Mϕ processes the arming factor by adding on a suppressor moiety, giving the factor the ability to directly suppress lymphocytes, perhaps by using an antigen bridge to locate their specific target cells. (*b*) Alternatively, the SAF could act as a specific receptor on the Mϕ which, when triggered by antigen, releases a locally reacting SIRS, which could give apparent specificity, again due to the antigen bringing the specific T cells into the local environment. Several workers have suggested that suppressor T cells may work by this indirect mechanism (2, 5, 15), and the evidence we have up to now strongly supports this possibility.[3] If further work confirms this point, it would be important to determine whether the SAF works to suppress the immune response because it only binds to Mϕ with a precommitted suppressor function, or whether all Mϕ have suppressor potential which the SAF can elicit or induce.

Lastly, the results described above give credence to, and help in the interpretation of, some previous work using HK Mϕ as competitive antagonists for L Mϕ functions (3–5). These studies indicated that HK Mϕ could interfere with

[2] W. Ptak, M. Zembala, and R. K. Gershon. Manuscript in preparation.

[3] W. Ptak, M. Zembala, M. Rewicka, and G. L. Asherson. Manuscript in preparation.

communications between T-cell subsets, and that the receptor on the Mϕ membrane which was responsible for the interference had Fc-like characteristics. It was further shown that these receptors on the HK Mϕ could adsorb helper as well as suppressor factors (4, 5). Since the HK Mϕ acted as competitive antagonists in a large number of (although clearly not all) immunological circumstances, it was suggested that the Mϕ serves an important role in the transmission of T-cell communication signals. The present results directly confirm this notion in at least one situation and, when considered in light of the cited previous work, indicate that the active biological role of Mϕ in the transmission of communication signals is not confined to this single suppressor system. It may be that the active Mϕ presentation of T-cell communication signals is as important to the immune response as is the active Mϕ presentation of antigen.

Summary

We have examined the ability of macrophages (Mϕ) to transmit T-cell derived suppressor signals to other T cells. The suppressor signal studied is an antigen-specific factor which suppresses the ability of adoptively transferred, sensitized lymphocytes to express contact hypersensitivity in normal recipients. We have found that this factor binds to peritoneal exudate Mϕ via cell surface structures which can be blocked with heat-aggregated gamma globulin. Dead (HK) Mϕ bind the factor but fail to present it in a functional way to assay (immune) T cells, whereas live (L) Mϕ perform both functions. Further, L Mϕ can retrieve the factor in an active form from the surfaces of HK Mϕ. Based on these and other findings (1–5), we discuss the possibility that Mϕ may play as important a role in presenting T-cell communication signals to the cells of the immune system as they do in presenting antigen.

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