Depression of Contact Sensitivity by *Pseudomonas aeruginosa*-Induced Suppressor Cells Which Affect the Induction Phase of Immune Response

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The cellular basis of depression of contact sensitivity to oxazolone in mice injected with *Pseudomonas aeruginosa* was studied. Cells from draining lymph nodes of mice sensitized with oxazolone 18 h previously were able to induce contact sensitivity to normal mice when administered in their footpads. In contrast, cells from draining lymph nodes of *P. aeruginosa*-injected and oxazolone-sensitized donors failed to induce contact sensitivity when injected in the footpad of normal mice and were capable of actively blocking the immunizing process brought about by lymph node cells from sensitized mice when injected together in the footpad of normal recipients. The *P. aeruginosa*-induced suppressor cells required antigenic stimulation, had precursors sensitive to cyclophosphamide, and did not affect the effector mechanisms of contact sensitivity. Thus, the results suggest that *P. aeurginosa* depresses contact sensitivity to oxazolone by enhancing the activity of suppressor cells which normally arise during the sensitization process and which affect the afferent limb of the immune response, probably by inhibiting the normal recruitment of T lymphocytes in the draining lymph nodes.

Several reports have focused attention on the immunodepressive capacity of *Pseudomonas aeruginosa* in humans and laboratory animals (7, 11, 19, 22). The mechanism(s) underlying this suppression, and its significance in the establishment and evolution of the infection, are largely undefined. We are currently using an experimental model of cell-mediated immunity, contact sensitivity to oxazolone in mice injected with *P. aeruginosa*, to investigate the cellular basis of this phenomenon. In previous studies we have shown that *P. aeruginosa* inhibits contact sensitivity to oxazolone by enhancing the activity of the suppressor cells which normally arise during the sensitization process (8, 10). On the other hand, it is known that several classes of suppressor cells, which occur after routine immunization, may regulate the response to contact-sensitizing agents in mice acting at different levels of the immune response, i.e., on the induction phase (1), during cell proliferation in the regional lymph nodes (4, 12, 21), and on the effector stage of contact sensitivity (23, 24).

The aim of this investigation was to determine what stage of the immune response was affected by *P. aeruginosa*-activated suppressor cells. The results suggest that, in mice sensitized with oxazolone, these cells interfere with the afferent limb of immune reaction by inhibiting the nor-

mal recruitment of T lymphocytes in the lymph nodes that drain the site of sensitization.

MATERIALS AND METHODS

Mice. Inbred C57BL/6 mice of both sexes raised in our institute and aged 8 to 12 weeks were used throughout. In each experiment the animals were randomly allocated to the different groups. Each group consisted of six or seven mice, unless otherwise stated.

Microorganism. A strain of *P. aeruginosa* isolated from clinical specimens was used. A stock culture on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) was transferred to a synthetic medium (17) and incubated with shaking and aeration at 37°C. After 24 h, the cells were collected, washed three times and then resuspended in distilled water, killed by heating at 65°C for 60 min, and then lyophilized.

Injection in mice. Mice were inoculated intravenously with 700 μg of heat-killed *P. aeruginosa* lyophilized cells suspended in 0.5 ml of sterile saline immediately before injection. This suspension contained approximately 1.2×10^{10} bacterial cells per ml. In a control trial for toxicity, no death was observed in injected mice over a period of 30 days.

Sensitization. Mice were anesthetized by intraperitoneal administration of tribromoethanol in amylene hydrate (Avertin; Winthrop Laboratories, New York, N. Y.) and sensitized for contact sensitivity 24 h after *P. aeruginosa* injection by painting the skin of the abdomen and lower thorax with 0.2 ml of 1.5% solution of 4-ethoxymethylene-2-phenyl-oxazolone (oxazolone;

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British Drug Houses, Poole, England) in absolute ethanol.

Measurement of DNA synthesis in draining lymph node cells. (i) In vitro. Brachial and inguinal lymph nodes from both control and *P. aeruginosa*-injected groups were removed on days 1, 3, 5, and 7 after sensitization, and a single-cell suspension was prepared. Triplicate cultures of 1×10^6 cells from each mouse in 0.2 ml of tissue culture medium RPMI-1640 (Gibco, Glasgow, Scotland) were set in a microtest culture plate and pulsed for 5 h with 1 μCi of [*methyl-*^{3}H]thymidine ([^{3}H]TdR) (The Radiochemical Centre, Amersham, England). The cells were then harvested and processed. Radioactivity was measured with a beta-spectrometer, and the counts were expressed as mean $\pm$ standard error of counts per minute.

(ii) In vivo. Incorporation of radiolabeled [^{125}I]5-iodo-2-deoxyuridine ([^{125}I]IUdR) (The Radiochemical Centre, Amersham, England) was used to measure regional lymph node cell proliferation according to the method of Pritchard and Micklem (14). On days 1, 3, 5, and 7 after oxazolone sensitization, four mice from both the control and the *P. aeruginosa*-injected groups were injected intraperitoneally with 5×10^{-8} mol of fluorodeoxyuridine in 0.2 ml of sterile distilled water. After 10 min, each animal received 1 μCi of [^{125}I]IUdR by the same route in the same volume. After 5 h, the mice were sacrificed and the brachial and inguinal lymph nodes were removed and placed in 1 ml of neutral buffered Formalin. Incorporation of [^{125}I]IUdR was determined by counting the tissues in a gamma-spectrometer and expressed as the mean $\pm$ standard error of the uptake percentage of [^{125}I]IUdR injected.

Induction of contact sensitivity by footpad transfer system. Donor mice were sensitized with oxazolone as described above. The regional brachial and inguinal lymph nodes were removed 18 to 24 h later and dissociated in Eagle minimal essential medium. The cells were washed twice, and their viability was tested by trypan blue dye exclusion. The stated number (see Results) of viable cells in 0.1 ml was injected into each mouse, and each hind footpad received 0.05 ml. After 6 days, the recipients were challenged by painting both sides of their ears with a drop of 1% oxazolone in olive oil. The quantification was made by measuring, with a micrometer, the increase in ear thickness 24 h later.

Passive transfer of contact sensitivity. Donor mice were sensitized with oxazolone on the abdomen and lower thorax as described above, and a total of 0.05 ml of 1.5% oxazolone solution was also applied to the four paws. Regional brachial and inguinal lymph nodes were removed after 3 days and dissociated in Eagle minimal essential medium. The cells were washed twice, and viability was tested by trypan blue dye exclusion. A total of 50×10^6 viable cells in 0.5 ml of medium was injected intravenously into normal recipients. The recipients were challenged within 2 h with 1% oxazolone in olive oil as described above.

Cyclophosphamide treatment. Cyclophosphamide (Endoxan Asta, Astawerke, AG, Chemische Fabrik, Brackwede, W. Germany), dissolved in sterile saline immediately before use, was injected intraperitoneally as a single dose of 200 mg/kg 2 days before sensitization.

Statistical analysis. Data are expressed as the mean $\pm$ standard error. Student's *t* test was used to compare the difference between the means.

RESULTS

Effect of *P. aeruginosa* on the DNA synthesis of draining lymph nodes in oxazolone-sensitized mice. Our previous results have shown that mice that were sensitized with oxazolone exhibited an impaired ear swelling after an epicutaneous challenge with the same antigen when injected intravenously with 700 μg of heat-killed *P. aeruginosa* 24 h before sensitization (10).

Moorhead (12) and Asherson and Barnes (2) have reported that, in mice painted on the skin with a contact-sensitizing agent, an increase in DNA synthesis and cell proliferation in the draining lymph nodes is induced and that this increase can already be detected 1 day after skin painting. Our first experiment was set up to investigate whether *P. aeruginosa* depressed contact sensitivity to oxazolone by interfering with the DNA synthesis and cell proliferation in the lymph nodes that drain the site of sensitization. A total of 64 C57BL/6 mice were divided into four groups. One group of animals was left untreated and used as a negative control, whereas two groups were injected intravenously with 700 μg of heat-killed *P. aeruginosa*. One of these two groups, together with the remaining group of mice, was sensitized with oxazolone 24 h later. On days 1, 3, 5, and 7 after sensitization, four mice of each of the four groups were sacrificed and the DNA synthesis of the draining lymph node cells was measured by pulsing the cells for 5 h in vitro with [^{3}H]TdR. The results reported in Fig. 1 show that DNA synthesis of the draining lymph node cells of mice that had been injected with *P. aeruginosa*, but not sensitized, was the same as that of untreated animals at all tested times. The DNA synthesis of the draining lymph node cells of mice sensitized, but not injected, with *P. aeruginosa* began to increase on day 1, reached a peak on day 3, and had declined to resting levels by day 5 after sensitization. In contrast, the [^{3}H]TdR uptake of draining lymph node cells of sensitized animals which had also been injected with *P. aeruginosa* was markedly reduced ($P < 0.005$) on day 3 after sensitization.

To ensure that the cell proliferation which was measured in vitro accurately reflected in vivo conditions, similar experiments were performed with [^{125}I]IUdR incorporation in vivo to measure cell proliferation in the lymph nodes that drain the site of sensitization, since it has been shown that [^{125}I]IUdR is rapidly incorporated into the DNA of proliferating cells (14).
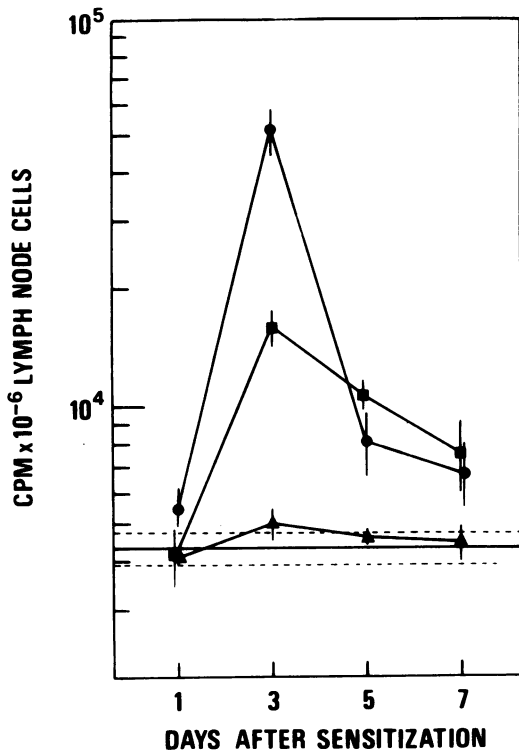
FIG. 1. *In vitro DNA synthesis, evaluated as [³H]TdR uptake and expressed as counts per minute (CPM), of 10⁶ brachial and inguinal lymph node cells at 1, 3, 5, and 7 days after oxazolone sensitization. ●, Oxazolone-sensitized mice; ■, P. aeruginosa-injected and oxazolone-sensitized mice; ▲, P. aeruginosa-injected but unsensitized mice. Horizontal lines refer to uninjected unsensitized mice. Data expressed as mean ± standard error.*

Thus, 80 C57BL/6 mice were divided into four groups. One group of animals was left untreated and used as a negative control, one group was injected with 700 μg of *P. aeruginosa*, one group was sensitized with oxazolone, and one group was both injected with 700 μg of *P. aeruginosa* and sensitized with oxazolone 24 h later. Cell proliferation in the draining lymph nodes was assessed on days 1, 3, 5, and 7 after sensitization by injecting five mice from each of the four groups with [¹²⁵I]IUdR. Figure 2 shows that the in vivo results parallel the in vitro ones. In fact, proliferation in the draining lymph nodes of mice which had been injected with *P. aeruginosa* but not sensitized was the same as that of untreated animals at all tested times. The [¹²⁵I]IUdR uptake, which in the untreated animals ranged from 0.026 to 0.040% of the injected radioactivity, rose in the uninjected sensitized mice to 0.190 ± 0.018% on day 3 and had declined to resting levels by day 5 after immunization. In

contrast, cell proliferation in the draining lymph nodes of sensitized animals which had also been injected with *P. aeruginosa* was lower at all tested times and reached its greatest depression on day 3 at 42% of cell proliferation in uninjected immunized mice ($P < 0.005$).

These findings raised the question of how *P. aeruginosa* could affect cell proliferation. The possibility of an altered reactivity of lymphocytes in mice injected with 700 μg of heat-killed *P. aeruginosa* was tested. A preparation of 6 × 10⁵ spleen or brachial and inguinal lymph node cells from normal mice or mice intravenously injected 4, 3, 2, or 1 day or 4 h previously with 700 μg of *P. aeruginosa* was incubated in 0.2 ml of culture medium RPMI-1640 for 36 h in either the presence or the absence of concanavalin A (0.6 μg/well) or *E. coli* 0.127:B₈ lipopolysaccharide (10 μg/well). The cultures were then pulsed with 0.5 μCi of radiolabeled TdR, and after a further 12 h incubation, the cells were harvested and processed. The results showed that the response of both spleen and lymph node cells of *P. aeruginosa*-injected mice did not differ from
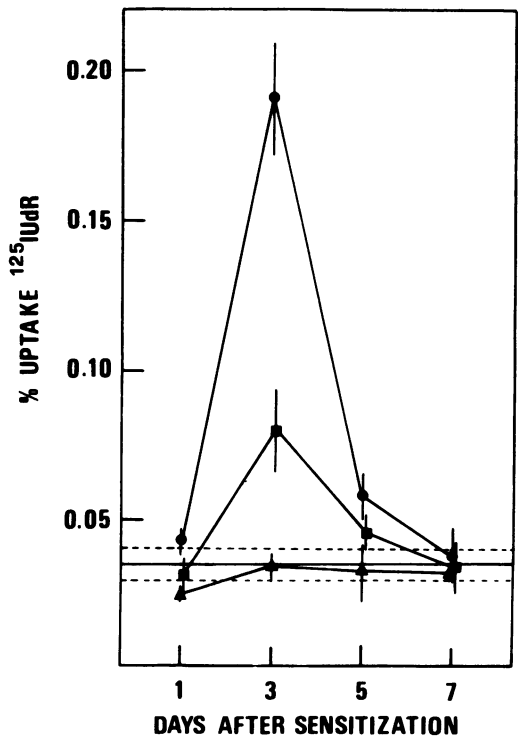

FIG. 2. *DNA synthesis, evaluated as percent uptake of [¹²⁵I]IUdR, of brachial and inguinal lymph nodes from mice sensitized 1, 3, 5, and 7 days previously with oxazolone. ●, Oxazolone-sensitized mice; ■, P. aeruginosa-injected and oxazolone-sensitized mice; ▲, P. aeruginosa-injected but unsensitized mice. Data expressed as mean ± standard error.*

that of uninjected controls at all tested times. These findings clearly indicate that this dose of bacteria does not exert a direct toxic effect on lymphocytes.

Footpad transfer of regional lymph node cells from *P. aeruginosa*-injected and oxazolone-sensitized mice in normal mice. Cell proliferation in the regional lymph nodes is the latest stage of the induction process of cell-mediated immune response. An early stage involves the collaboration between a particular subset of T cells (initiator T cells), which possess the antigen in an immunizing form, and a second class of T cells (recruited T cells) which proliferate and become the effector cells of the immune response (9, 18). This mechanism is also operative in the contact sensitivity model. Recently, it has been shown that a small number of cells taken from the draining lymph nodes of mice one day after sensitization with a contact-sensitizing agent and injected into the footpad of normal recipients are able to recruit recipient T lymphocytes and so induce contact sensitivity in the host. This is an immunizing process, rather than a passive transfer of sensitized cells (6, 20).

By using the footpad transfer system described by Asherson et al. (6), we first investigated whether lymph node cells from *P. aeruginosa*-injected and oxazolone-sensitized mice were able to induce contact sensitivity in normal animals. Thus, normal mice were injected with 2×10^6, 4×10^6, or 8×10^6 cells from draining lymph nodes of either syngeneic donors which had been sensitized with oxazolone 24 h previously or donors which had also been injected with *P. aeruginosa* 24 h before sensitization. The recipients were challenged 6 days after cell transfer by painting both sides of their ears with a drop of oxazolone in olive oil. The results (Fig. 3) show that ear swelling was lower in mice receiving 4×10^6 or 8×10^6 cells from donors that had been both *P. aeruginosa*-injected and oxazolone-sensitized than in mice receiving the same number of cells from only sensitized donors ($P < 0.05$, at least). In the case of transfer of 2×10^6 cells, the difference did not reach statistical significance, probably owing to the small degree of contact sensitivity induced. These results indicate that the draining lymph node cells of *P. aeruginosa*-injected and oxazolone-sensitized mice fail to induce contact sensitivity in the normal host.

Suppressor cells in the draining lymph nodes of *P. aeruginosa*-injected and oxazolone-sensitized mice. The above findings that draining lymph node cells from mice which had been both *P. aeruginosa* injected and oxazolone sensitized failed to induce contact sensitivity in
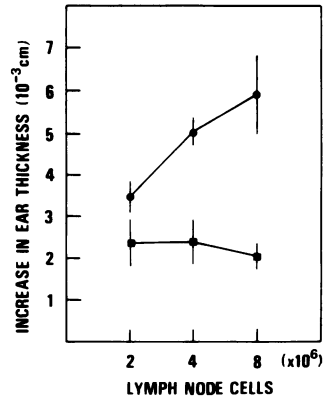

FIG. 3. *Induction of contact sensitivity by footpad injection of various numbers of brachial and inguinal lymph node cells from either oxazolone-sensitized donors (●) or P. aeruginosa-injected and oxazolone-sensitized donors (■). The challenge was performed 6 days after cell transfer, and contact sensitivity was expressed as increase in ear thickness 24 h later in units of 10^{-3} cm. Data expressed as mean ± standard error.*

normal animals when transferred into their footpads, together with our previous results showing that suppressor cells occur in *P. aeruginosa*-injected and oxazolone-sensitized mice (10), seem to suggest that the *P. aeurginosa*-activated suppressor cells may occur early in the sensitization process in the draining lymph nodes, thus blocking the induction stage of contact sensitivity. To directly test this hypothesis, we again used the footpad transfer system. One group of normal mice was injected with 4×10^6 cells from draining lymph nodes of syngeneic animals sensitized with oxazolone 24 h previously; three groups of mice were injected in their footpads with the same number of immunizing cells plus 2×10^6, 4×10^6, and 8×10^6 cells, respectively, from donors sensitized with oxazolone 24 h previously and also injected with *P. aeruginosa* 24 h before sensitization; one group of untreated mice was the negative control. The recipients were challenged 6 days after cell transfer.

The results (Table 1) show that 2×10^6, 4×10^6, or 8×10^6 cells from donors which had been both injected with *P. aeruginosa* and sensitized with oxazolone were all able to depress the ear swelling of animals immunized with 4×10^6 cells from only sensitized donors. This finding indicates that the lymph nodes of *P. aeruginosa*-injected and oxazolone-sensitized mice contain cells that are capable of suppressing the induction of contact sensitivity. Since in normal animals the immunizing effect of cells from oxazolone-sensitized donors is inhibited by cells from *P. aeruginosa*-injected and oxazolone-sensitized

TABLE 1. Contact sensitivity of mice receiving in their footpads 4 × 10⁶ lymph node cells from oxazolone-sensitized donors and varying numbers of lymph node cells from P. aeruginosa-injected and oxazolone-sensitized donors

Cells injected[a] (× 10⁶)	Contact sensitivity[b]	P[c]
4 LN (Ox)	4.56 ± 0.46	
4 LN (Ox) + 2 LN (PA Ox)	1.80 ± 0.63	<0.01
4 LN (Ox) + 4 LN (PA Ox)	1.17 ± 0.37	<0.001
4 LN (Ox) + 8 LN (PA Ox)	1.62 ± 0.38	<0.001
	0.78 ± 0.11	

[a] LN, lymph node cells; (Ox), from oxazolone-sensitized mice; (PA Ox), from P. aeruginosa-injected and oxazolone-sensitized mice.

[b] Expressed as increase in ear thickness 24 h after challenge in units of 10^{-3} cm. All data are expressed as mean ± standard error.

[c] Level of significance when compared with the control group.

mice, these results also suggest that the target of P. aeruginosa-activated suppressor cells seems to be the collaboration between donor "initiator" T cells (or macrophages) and recipient "recruited" T cells.

Antigen requirement and cyclophosphamide sensitivity of P. aeruginosa-induced suppressor cells. In the next series of experiments, we studied some characteristics of the P. aeruginosa-activated suppressor cells, such as their antigen requirement and their sensitivity to cyclophosphamide, a drug known to enhance delayed hypersensitivity reactions by eliminating the precursors of suppressor cells (13, 16, 23). For this purpose, one group of normal mice was immunized by footpad injection of 4 × 10⁶ lymph node cells from oxazolone-sensitized mice and was the positive control. Three experimental groups consisted of mice injected into the footpad with the same number of immunizing cells plus (i) 4 × 10⁶ lymph node cells from P. aeruginosa-injected and oxazolone-sensitized mice, (ii) 4 × 10⁶ lymph node cells from P. aeruginosa-injected but unsensitized mice, and (iii) 4 × 10⁶ lymph node cells from cyclophosphamide-pretreated, P. aeruginosa-injected, and oxazolone-sensitized mice, respectively. A fourth experimental group of normal recipients was injected only with 4 × 10⁶ lymph node cells from cyclophosphamide-pretreated, P. aeruginosa-injected, and oxazolone-sensitized donors. One group of untreated mice was the negative control. The recipients were challenged 6 days later.

The results (Table 2) show that only lymph node cells from either P. aeruginosa-injected but unsensitized mice or cyclophosphamide-pretreated, P. aeruginosa-injected, and oxazolone-sensitized mice were unable to suppress the in-

duction of contact sensitivity brought about by lymph node cells from oxazolone-sensitized donors. Furthermore, the ear swelling of animals receiving cells from cyclophosphamide-pretreated, P. aeruginosa-injected, and oxazolone-sensitized donors did not differ from that of the positive control group. These findings, in agreement with our previous results (10), indicate that the P. aeruginosa-induced suppressor cells require antigenic stimulation and have cyclophosphamide-sensitive precursors.

Inability of lymph node cells from P. aeruginosa-injected and oxazolone-sensitized mice to suppress the effector stage of contact sensitivity. To test whether P. aeruginosa-induced suppressor cells were active on the effector phase of contact sensitivity, as well as on the inductive stage, we used cell transfer experiments. Draining lymph node cells from P. aeruginosa-injected and oxazolone-sensitized mice, taken 3 days after sensitization, were used as the suppressor cell source on the basis of our previous results (10). Thus, three groups of normal mice were sensitized with oxazolone. The first group, which received no cells, was the control group. Within 2 h after sensitization, the second group was intravenously injected with 50 × 10⁶ cells from donors sensitized with oxazolone 3 days previously, and the third group received the same number of cells from donors sensitized 3 days previously and also injected with P. aeruginosa 24 h before sensitization. The same number of cells from the same two groups of donor mice was also intravenously transferred into two groups of mice, sensitized 6 days previously, 2 h before their challenge. The challenge of the control and the experimental groups was performed 6 days after their respective sensitization.

As can be seen in Table 3, the ear swelling of

TABLE 2. Contact sensitivity of mice receiving in their footpads 4 × 10⁶ lymph node cells from oxazolone-sensitized donors and 4 × 10⁶ lymph node cells from donors treated in different ways

Cells injected[a] (× 10⁶)	Contact sensitivity[b]	P[c]
4 LN (Ox)	4.95 ± 0.89	
4 LN (Ox) + 4 LN (PA Ox)	2.17 ± 0.35	<0.001
4 LN (Ox) + 4 LN (PA)	5.05 ± 0.28	NS
4 LN (Ox) + 4 LN (CY PA Ox)	4.21 ± 0.32	NS
4 LN (CY PA Ox)	5.50 ± 1.06	NS
	0.81 ± 0.28	

[a] LN, lymph node cells; (Ox), from oxazolone-sensitized mice; (PA Ox), from P. aeruginosa-injected and oxazolone-sensitized mice; (PA), from P. aeruginosa-injected but unsensitized mice; (CY PA Ox), from cyclophosphamide-pretreated, P. aeruginosa-injected, and oxazolone-sensitized mice.

[b] See Table 1, footnote b.

[c] See Table 2, footnote c. NS, Not significant.

mice receiving lymph node cells from oxazolone-sensitized mice either at the moment of sensitization or at the moment of challenge did not differ from that of the control group. On the contrary, the response to oxazolone of mice receiving cells from donors which had been both *P. aeruginosa* injected and oxazolone sensitized was significantly ($P < 0.001$) depressed in the group of animals which received these cells at the moment of sensitization, but it was not altered in the animals in which cell transfer was performed at the moment of challenge.

In mice sensitized with a contact-sensitizing agent it has been found that, 3 to 4 days after sensitization, the draining lymph nodes and spleens contain cells able to passively transfer contact sensitivity to normal mice. These cells, sometimes called "passive transfer cells," are the effector cells of contact sensitivity (3, 15). To confirm that *P. aeruginosa*-activated suppressor cells were unable to affect the effector stage of contact sensitivity, we used a passive transfer system of contact sensitivity. Two groups of donor mice were used: one group was injected intravenously with heat-killed *P. aeruginosa*, and the other was left untreated; 24 h later, both groups were sensitized with oxazolone. The two groups, together with a group of normal mice, were sacrificed 3 days after sensitization. Preparations of 50×10^6 normal lymph node cells plus 50×10^6 lymph node cells from oxazolone-sensitized donors were transferred intravenously into one group of normal recipients. Another group of normal recipients received 50×10^6 normal lymph node cells plus 50×10^6 lymph node cells from *P. aeruginosa*-injected and oxazolone-sensitized donors. A third group of normal mice were given both 50×10^6 cells from oxazolone-sensitized mice (passive transfer cells) and 50×10^6 cells from *P. aeruginosa*-injected and oxazolone-sensitized mice (suppressor cells).

The recipients were challenged within 2 h after cell transfer.

The results show that lymph node cells from *P. aeruginosa*-injected and oxazolone-sensitized mice failed to transfer contact sensitivity to normal animals and were unable to block the response to oxazolone brought about by passive transfer cells (Table 4).

DISCUSSION

In a previous paper we showed that the depression of contact sensitivity in *P. aeruginosa*-injected and oxazolone-sensitized mice seems to be due to the activation of the specific suppressor cells which normally arise in the draining lymph nodes and spleen during the sensitization process (10). The present report provides evidence that the *P. aeruginosa*-activated suppressor cells in mice sensitized with oxazolone exert their effect very early in the induction phase of contact sensitivity.

In fact, *P. aeruginosa*-activated suppressor cells (i.e., lymph node cells from mice which had been injected with *P. aeruginosa* 4 days previously and sensitized with oxazolone 24 h later) did not affect the expression of contact sensitivity brought about by the passive transfer cells (i.e., lymph node cells from mice which had been sensitized only 3 days previously) when they were transferred together into normal syngeneic recipients. In other words, the *P. aeruginosa*-activated suppressor cells did not inhibit the efferent limb of the immune reaction. It was also found that these suppressor cells had no effect on the ear swelling of sensitized recipients when administered intravenously immediately before the recipient challenge, although these cells were able to depress the response to oxazolone of normal recipients when transferred at the time of recipient sensitization. This finding confirms that, in our model, the suppressor cells do not affect the effector mechanisms of contact sensitivity and also indicates that they exert their effect on the afferent side of immune response.

It has been well established that, after im-

TABLE 3. *Contact sensitivity of sensitized mice receiving intravenously lymph node cells from P. aeruginosa-injected and oxazolone-sensitized donors either after sensitization or before challenge*

Cells transferred within 2 h after sensitization[a] (50×10^6)	Cells transferred 2 h before challenge[a] (50×10^6)	Contact sensitivity[b]
		9.15 ± 1.25
LN (Ox)		8.60 ± 0.77
LN (PA Ox)		3.88 ± 0.47^c
	LN (Ox)	11.00 ± 1.12
	LN (PA Ox)	8.40 ± 0.20

[a] See Table 1, footnote a.
[b] See Table 1, footnote b.
[c] Level of significance when compared with the control group receiving no cells, $P < 0.001$.

TABLE 4. *Inability of P. aeruginosa-induced suppressor cells to affect in normal recipients the expression of contact sensitivity brought about by lymph node cells from sensitized donors*

No. of transferred cells[a] (50×10^6)	Contact sensitivity[b]	P[c]
LN (Ox) + LN (−)	4.62 ± 0.57	
LN (PA Ox) + LN (−)	1.28 ± 0.54	<0.005
LN (Ox) + LN (PA Ox)	4.48 ± 0.64	NS

[a] See Table 1, footnote a. (−), From normal mice.
[b] See Table 1, footnote, b.
[c] See Table 1, footnote c. NS, Not significant.

munization with a contact sensitivity agent, the antigen is taken up by macrophages and transferred either directly or via a special class of radioresistant T cells, called "initiator" T cells, to another class of T cells ("recruited" T cells) (6, 20). The latter cells proliferate in the draining lymph nodes and spleen, leave these organs after 3 to 4 days, and settle in the bone marrow, where they continue to divide and provide the cells which maintain contact sensitivity (5).

Since our results have shown that the immunizing effect brought about in normal animals by cells from oxazolone-sensitized donors is inhibited by cells from *P. aeruginosa*-injected and oxazolone-sensitized mice, the primary target of the *P. aeruginosa*-activated suppressor cells seems to be the collaboration between the initiator T cells or macrophages and recruited T cells. Thus, a defective activation of recruited T lymphocytes could account for the decrease in cell proliferation observed in the draining lymph nodes of sensitized mice which had also been injected with *P. aeruginosa*.

The response to a contact sensitivity agent is known to be regulated by a complex and specific suppressor system. In fact, lymph nodes and spleens of mice painted on the skin with a contactant contain (i) B suppressor cells, which appear from the day 6 day after sensitization onwards, acting on the effector stage of contact sensitivity (23, 24); (ii) B suppressor cells which depress the induction of contact sensitivity (1); and (iii) T suppressor cells which depress the in vivo DNA synthesis response to the contactant (4, 12, 21).

The sensitivity to cyclophosphamide of the cells responsible for the depression of contact sensitivity to oxazolone in *P. aeruginosa*-injected mice, their early presence in the draining lymph nodes, and their ability to affect a very early stage of contact sensitivity suggest that these cells could be the B suppressor cells, described by Asherson, which depress the induction of contact sensitivity, probably by limiting the persistence of the antigen in an immunizing form on macrophages and/or initiator T cells (1). However, experiments are now being undertaken to answer a number of questions regarding the *P. aeruginosa*-activated suppressor cells, i.e., the inducing stimulus, their nature, and exactly how their effect is exerted.

LITERATURE CITED

1. Asherson, G. L. 1977. Depression of cell-mediated immunity by pretreatment with adjuvants, p. 382–387. *In* D. Schlessinger (ed.), Microbiology—1977. American Society for Microbiology, Washington, D.C.

2. Asherson, G. L., and R. M. R. Barnes. 1973. Contact sensitivity in the mouse. XII. The use of DNA synthesis *in vivo* to determine the anatomical location of immunological unresponsiveness to picryl chloride. Immunology 25:495–508.

3. Asherson, G. L., and W. Ptak. 1968. Contact and delayed hypersensitivity in the mouse. I. Active sensitization and passive transfer. Immunology 15:405–416.

4. Asherson, G. L., P. J. Wood, and B. Mayhew. 1975. Control of the immune response. I. Depression of DNA synthesis by immune lymph node cells. Immunology 29:1057–1065.

5. Asherson, G. L., and M. Zembala. 1973. Anatomical location of cells which mediate contact sensitivity in the lymph nodes and bone marrow. Nature (London) New Biol. 244:176–177.

6. Asherson, G. L., M. Zembala, and B. Mayhew. 1977. Analysis of the induction phase of contact sensitivity by footpad transfer of regional lymph node cells: macrophages and radioresistant T lymphocytes to induce immunity. Immunology 32:81–88.

7. Campa, M., C. Garzelli, and G. Falcone. 1975. Depression of contact sensitivity and enhancement of antibody response in *Pseudomonas aeruginosa*-infected mice. Infect. Immun. 12:1252–1257.

8. Campa, M., C. Garzelli, E. Ferrannini, and G. Falcone. 1976. Evidence for suppressor cell activity associated with depression of contact sensitivity in *Pseudomonas aeruginosa* infected mice. Clin. Exp. Immunol. 26:355–362.

9. Cohen, I. R., S. Livnat, and S. D. Waksal. 1978. Initiator and recruited T lymphocytes are distinct subclasses of T lymphocytes. Eur. J. Immunol. 8:35–41.

10. Colizzi, V., C. Garzelli, M. Campa, and G. Falcone. 1978. Depression of contact sensitivity by enhancement of suppressor cell activity in *Pseudomonas aeruginosa*-injected mice. Infect. Immun. 21:354–359.

11. Floersheim, G. L., W. H. Hopff, M. Gasser, and K. Bucker. 1971. Impairment of cell-mediated immune responses by *Pseudomonas aeruginosa*. Clin. Exp. Immunol. 9:241–247.

12. Moorhead, J. W. 1976. Tolerance and contact sensitivity to DNFB in mice. VI. Inhibition of afferent sensitivity by suppressor T cells in adoptive tolerance. J. Immunol. 117:802–806.

13. Polak, L., and J. L. Turk. 1974. Reversal of immunological tolerance by cyclophosphamide through the inhibition of suppressor cell activity. Nature (London) 249:654–656.

14. Pritchard, H., and H. S. Micklem. 1972. Immune responses in congenitally thymus-less mice. I. Absence of response to oxazolone. Clin. Exp. Immunol. 10:151–161.

15. Ptak, W., and G. L. Asherson. 1969. Contact and delayed hypersensitivity in the mouse. II. The role of different cell populations. Immunology 17:769–775.

16. Shand, F. L., and J. G. Howard. 1978. Cyclophosphamide inhibited B cell receptor regeneration as a basis for drug-induced tolerance. Nature (London) 271:255–257.

17. Stayner, R. J., N. J. Palleroni, and N. Doudoroff. 1968. The aerobic *Pseudomonads*: a taxonomic study. J. Gen. Microbiol. 43:159–271.

18. Steinman, L., E. Tzehoval, I. R. Cohen, S. Segal, and E. Glickman. 1978. Sequential interaction of macrophages, initiator T lymphocytes and recruited T lymphocytes in a cell-mediated immune response to a soluble antigen. Eur. J. Immunol. 8:29–34.

19. Stone, H. H., K. S. Given, and J. D. Martin. 1967. Delayed rejection of skin homografts in *Pseudomonas* sepsis. Surg. Gynecol. Obstet. 124:1067–1070.

20. Thomas, W. R., G. L. Asherson, and M. A. C. C.

Perera. 1978. Induction of contact sensitivity. Selective induction of delayed hypersensitivity by the injection of cells from draining lymph nodes into the footpads of normal recipients. Immunology **34**:725-731.

21. **Wood, P., G. L. Asherson, B. Mayhew, W. R. Thomas, and M. Zembala.** 1977. Control of the immune reaction: T cells in immunized mice which depress the *in vivo* DNA synthesis response in the lymph nodes to skin painting with the contact sensitizing agent picryl chloride. Cell Immunol. **30**:25-34.

22. **Woodruff, M. F. A., B. Nolan, J. S. Robson, and M. K.** MacDonald. 1969. Renal transplantation in man. Lancet i:6-12.

23. **Zembala, M., and G. L. Asherson.** 1976. The effect of cyclophosphamide and irradiation on cells which suppress contact sensitivity in the mouse. Clin. Exp. Immunol. **23**:554-561.

24. **Zembala, M., G. L. Asherson, J. Noworolski, and B. Mayhew.** 1976. Contact sensitivity to picryl chloride: the occurrence of B suppressor cells in the lymph nodes and spleen of immunized mice. Cell. Immunol. **25**:266-278.