Inhibition of Proliferative Responses and Interleukin 2 Productions by Salazosulfapyridine and Its Metabolites

Masami FUJIWARA, Kazutaka MITSUI and Itaru YAMAMOTO*

Department of Immunochemistry, Faculty of Pharmaceutical Sciences, Okayama University, Tsusima-naka 1-1-1, Okayama 700, Japan

Accepted June 7, 1990

Abstract—Mixed lymphocytes reactions (MLR) and concanavalin A (Con A)- or phytohemagglutinin (PHA)-stimulated proliferative responses were dose-dependently inhibited by salazosulfapyridine (SASP) and cyclosporin A (CsA) in the concentration ranges of $1 \times 10^{-5}$-5 $\times 10^{-4}$ M and 10-1000 ng/ml, respectively. Such a significant inhibition was not observed with metabolites of SASP, sulfapyridine (SP) and 5-aminosalicylic acid (5-ASA). In addition, SASP and CsA inhibited the production of interleukin 2 (IL-2) from splenocytes in these experiments. The inhibitory effect of CsA on IL-2 production practically correlated with that on proliferative responses, whereas SASP showed a less marked inhibitory effect on IL-2 production than on proliferative responses. Neither SP nor 5-ASA inhibited the IL-2 production. In the Con A-induced proliferative response, SASP showed a full inhibition even when added after 4-8 hr of culture, but CsA did not. The splenocytes that were pulsed with Con A for 4 hr could proliferate in response to Con A-supernatant or purified IL-2. CsA exhibited the inhibitory activity only when present during the time of Con A-pulsing, while SASP acted on the subsequent stage of the response, exerting its inhibitory effect. These findings suggest that SASP down-regulates the immune response by a mechanism apparently distinct from that of CsA.

Salazosulfapyridine (SASP) was first reported by Svartz (1) to be effective in the treatment of rheumatoid arthritis (RA). A high incidence of adverse effects such as nausea, anorexia, dispesia and abdominal pain was reported by Sinclair and Duthie (2); since then, SASP has been used for maintenance therapy of ulcerative colitis rather than for RA. Thereafter, McConkey et al. (3) reevaluated the effectiveness of SASP in RA and papers appeared describing that SASP has an anti-rheumatic effect comparable to those of D-penicillamine and gold compounds (4, 5). Moreover, the effect of SASP was evaluated in experiments using animal models (6, 7), but the mechanism underlying the anti-rheumatic effect of SASP remains unclear. Accordingly, it is important to elucidate the immunopharmacological action of SASP.

We have already reported that SASP has a marked inhibitory effect on in vitro production of antibodies, suggesting that its inhibitory effect was mediated by T cells (8). In addition, it was found that interleukin 2 (IL-2) production stimulated with sheep red blood cells (SRBC) was also inhibited by SASP. Recently, we have found that SASP inhibits the anti-DNA antibody production in autoimmune mice (manuscript is in preparation).

Cyclosporin A (CsA), a fungal cyclic polypeptide, is an immunosuppressive agent used as a rejection inhibitor in organ transplantation, which acts principally by inhibiting T cell functions. In particular, much information is available concerning the effect of CsA on IL-2 production and IL-2 receptor (R) expression (9-13). In this study, the effects of SASP on the proliferation and IL-2 production induced by alloantigen, concanavalin A (Con A) or phytohemagglutinin (PHA), in
which T cells are the dominant responder, were investigated and compared with those of CsA. Additionally, the major metabolites of SASP, sulfapyridine (SP) and 5-aminosalicylic acid (5-ASA), were also evaluated for their effects.

Materials and Methods

Animals: The BALB/c mice and C57BL/6 mice (female, used at 8–10 weeks of age) were purchased from Charles River, Inc.

Reagents: Reagents used were obtained from the following sources: Con A, Sigma; PHA-P, DIFCO; fetal calf serum (FCS), Cell Culture Technology; [6-3H]thymidine (3H-TdR), Amersham International. RPMI-1640 medium (Flow Laboratories) was supplemented with penicillin G (100 units/ml) and streptomycin (100 μg/ml). L-Glutamine (Tokyo Chemical Industry) at 2 mM was added to the culture medium only for the mixed lymphocytes reactions (MLR). SASP, SP and 5-ASA (Pharmacal LEO Therapeutics AB) were dissolved in RPMI-1640 medium containing NaOH (the final concentration was \(3.3 \times 10^{-4}\) N). CsA (Sandoz) was dissolved in RPMI-1640 medium containing dimethyl sulphoxide (DMSO, the final concentration was 0.025% v/v) as described by Bunjes et al. (9). Each drug was diluted and added to the cultures. Prior to this study, these solvents were ascertained not to influence relevant reactions and cellular survivals.

MLR: Spleen cells (5\(\times\)10⁵) from BALB/c mice as responders and X-ray (3000 rad) irradiated spleen cells (5\(\times\)10⁵) from C57BL/6 mice as stimulators were co-cultured for 3 days with varying concentrations of test drugs in 0.2 ml of 10% FCS-RPMI-1640 medium using 96-well flat-bottomed microculture plates (NUNC) and pulsed with 3H-TdR (18.5 kBq) for an additional 18 hr. The cells were harvested onto glass fiber filters, and the incorporated radioactivity was measured with a liquid scintillation counter. Moreover, the activity of IL-2 in the culture supernatant on day 2 was measured by the method described below.

Con A- and PHA-response: Con A- and PHA-induced proliferative responses were determined as follows. BALB/c mouse spleen cells (2\(\times\)10⁵) were cultured for 2 days with an optimal concentration of Con A (5 μg/ml) or PHA (50 μg/ml) in the presence of varying concentrations of test drugs in 0.2 ml of 5% FCS-RPMI-1640 medium using 96-well round-bottomed microculture plates (NUNC). Cultures were pulsed with 3H-TdR (18.5 kBq) for 18 hr, and the radioactivity incorporated into cells was measured as described above. For IL-2 production, spleen cells (5\(\times\)10⁵) were incubated with Con A (5 μg/ml) or PHA (100 μg/ml) in the presence of varying concentrations of test drugs in 1 ml of 5% FCS-RPMI-1640 medium using 24-well multidishes (NUNC). After 24 hr, the supernatants were collected and assayed for IL-2 activity.

The procedure for removing Con A from spleen cells after a Con A-pulse was as follows: Spleen cells were stimulated with Con A for 4 hr and harvested. After being washed, they were incubated for 30 min in minimum essential medium (MEM, Nissui Pharmaceutical Co., Ltd.) containing 20 mg/ml methyl-α-D-mannopyranoside (α-MM, Sigma), 10 mM HEPES (Sigma) and 10% FCS, and then washed in three changes of MEM. The resulting cells were recultured in the presence of 50% v/v Con A-supernatant (CAS) or 100 U/ml human ultrapure IL-2 (GUPI-2, Genzyme), and the proliferative response was determined. CAS was prepared as described by Larsson and Conutinho (14). Briefly, BALB/c mouse spleen cells (5\(\times\)10⁶/ml) were cultured with Con A (5 μg/ml) in 10% FCS-1640 medium using a culture flask (25 cm², 50 ml, NUNC). After 20 hr, the supernatant, CAS, was withdrawn and added with 20 mg/ml α-MM to neutralize Con A activity. Because the proliferative response of spleen cells could not be induced by CAS alone, Con A in CAS was ascertained to be inactivated. The CAS so prepared was stored at −20°C until use.

IL-2 assay: IL-2 activity of culture supernatants was measured by bioassay using CTLL-2 cells (supplied by Dr. K. Kohno, Hayashibara Biochemical Laboratories, Inc.), an IL-2 dependent murine cytotoxic T cell line (15). The assay was carried out according to Kohno et al. (16). Briefly, two-fold serial dilutions of culture supernatants were added.
to the culture of CTLL-2 (4x10^3) for 24 hr in a 96-well flat-bottomed microculture plate. They were pulsed with ³H-TdR (9.25 kBq) for the last 6 hr, and the radioactivity incorporated into CTLL-2 cells was determined. The IL-2 activity yielding 50% of the maximum ³H-TdR uptake of standard IL-2 preparation was defined as one unit according to the method of Gillis et al. (17). Because the IL-2 activity of culture supernatants from cells stimulated with alloantigen or PHA was low and its activity could not be defined in terms of units, it was expressed by the CTLL-2 cell proliferation induced by a test sample in an appropriate dilution.

IL-2 R assay: According to the method of Altman et al. (18), the IL-2 R expression of Con A-activated cells was indirectly detected by determining the ability of activated splenocytes to adsorb IL-2. Briefly, 5x10^6 spleen cells from BALB/c mice cultured for 24 hr with Con (5 µg/ml) in the presence or absence of SASP or CsA were harvested.

After being washed, they were incubated for 30 min. This procedure was repeated one more time. The resulting cells were resuspended in 1 ml of appropriately diluted culture supernatant (including IL-2 activity) obtained from BALB/c mouse splenocytes stimulated with Con A for 24 hr and supplemented with 20 mg/ml α-MM, and incubated for 2 hr with occasional shaking. After centrifugation, the residual IL-2 activity of the resulting supernatant was assayed by the above-mentioned method.

Statistical analysis: All results are expressed as the arithmetic mean±S.D. of triplicate sets of a typical experiment in several independent studies. Statistical significance was analyzed by Student's t-test.

Results

Effects of SASP, SP, 5-ASA and CsA on proliferative response and IL-2 production in MLR: As shown in Fig. 1A, SASP inhibited the proliferative response in MLR in a dose-
dependent manner with inhibition rates of 45% at 1×10⁻⁴ M, 86% at 2×10⁻⁴ M and 98% at 5×10⁻⁴ M. SP, one of the major metabolites of SASP, failed to suppress this proliferative response, and the other metabolite 5-ASA had a weak inhibitory effect. Consistent with the previous report by Hess et al. (11), CsA produced a dose-dependent inhibitory effect in the concentration range of 10 to 1000 ng/ml, as shown by its inhibition rate of 66% at 100 ng/ml and 88% at 1000 ng/ml (Fig. 1B). Although no data was given here, the production of IL-2 in MLR reached a maximum on day 2 during the culture period, decreasing thereafter. Accordingly, effects of these agents on IL-2 released into the supernatants on day 2 was studied. The IL-2 production was reduced 18%, 29% and 84% by 1×10⁻⁴ M, 2×10⁻⁴ M and 5×10⁻⁴ M of SASP, respectively (Fig. 2A). No significant inhibition was observed by SP or 5-ASA.

CsA, an IL-2 inhibitor, significantly inhibited IL-2 production by 46% at 100 ng/ml and 95% at 1000 ng/ml (Fig. 2B). Any of SASP, SP, 5-ASA and CsA at the doses used in the present study was ascertained not to directly influence IL-2-dependent proliferation of CTLL-2 cells, although the data are not shown here.

Effects of SASP and CsA on Con A- and PHA-induced proliferative responses and IL-2 production: SASP and CsA were also studied to determine their effects on the proliferative responses induced by Con A and PHA, T cell mitogenets. The response was almost completely inhibited by SASP at a concentration of 2×10⁻⁴ M or more (Fig. 3A). The metabolites SP and 5-ASA at a concentration of 5×10⁻⁴ M or less had hardly any effect (data not shown). CsA also showed a dose-dependent inhibitory effect on Con A- and PHA-induced proliferative responses in

![Graph showing the effects of SASP, SP, 5-ASA, and CsA on IL-2 production in MLR.](image-url)
Fig. 3. Effects of SASP and CsA on the Con A- or PHA-induced proliferative responses. BALB/c mouse spleen cells (2×10^6) were cultured with various concentrations of SASP (A) or CsA (B) in the presence of Con A (5 μg/ml, —) or PHA (50 μg/ml, ---) for 48 hr and pulsed with ^3^H-TdR for an additional 18 hr. Results are the mean±S.D. of triplicate sets of a typical experiment in several independent studies. *: P<0.05, **: P<0.01, as compared with the respective control value.

Table 1. Effects of SASP and CsA on the Con A-stimulated IL-2 production

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration</th>
<th>IL-2 activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>U/ml</td>
</tr>
<tr>
<td>Exp. 1</td>
<td>SASP (M)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1×10^-4</td>
<td>388</td>
</tr>
<tr>
<td></td>
<td>2×10^-4</td>
<td>350</td>
</tr>
<tr>
<td></td>
<td>5×10^-4</td>
<td>294</td>
</tr>
<tr>
<td></td>
<td>CsA (ng/ml)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>SASP (M)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2×10^-4</td>
<td>184</td>
</tr>
<tr>
<td></td>
<td>5×10^-4</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>CsA (ng/ml)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>158</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>32</td>
</tr>
</tbody>
</table>

BALB/c mouse spleen cells (5×10^6/well) were cultured with varying concentrations of SASP or CsA in the presence of Con A (5 μg/ml). Supernatants were collected at 24 hr and then assayed for their ability to support growth of the IL-2 dependent CTLL-2 cells (see Materials and Methods). Maximum ^3^H-TdR uptakes of CTLL-2 cells induced by the standard IL-2 preparation were 23,636±683 (Exp. 1) and 33,177±2593 (Exp. 2). The results are representative of several similar experiments.
the concentration range of 10 to 1000 ng/ml (Fig. 3B). Next we studied the effects of two drugs on IL-2 production stimulated with Con A (Table 1) or PHA (Fig. 4), in which the maximum responses were observed 24 hr after the stimulation. SASP at concentrations of $2 \times 10^{-4}$ M and $5 \times 10^{-4}$ M, which produced more than 90% inhibition of the proliferative responses induced by Con A or PHA, inhibited IL-2 production by only 20-40% (Table 1). CsA at 100 ng/ml and 1000 ng/ml caused more than 90% inhibition. In the PHA-induced IL-2 production, as observed in the Con A-stimulation, the inhibitory action of SASP was weaker than that of CsA (Fig. 4).

**Effect of SASP and CsA on Con A-stimulated expression of IL-2 R:** The IL-2 R expression of spleen cells activated by Con A was determined by the ability of the cells to adsorb IL-2 activity. That is, 24 hr after the stimulation with Con A in the presence or absence of SASP or CsA, these activated cells were incubated with the IL-2 preparation and the residual IL-2 activity was titrated. As shown in Fig. 5, the level of IL-2 adsorption was decreased in cells cultured in the presence of SASP ($2 \times 10^{-4}$ M) or CsA (100 ng/ml), as compared with that for control cells, suggesting that both compounds inhibited IL-2 R expression. The inhibitory effect was more prominent for CsA than for SASP.

**Kinetic study of inhibitory effects of SASP and CsA on Con A-induced proliferative response:** Both SASP and CsA showed a dose-dependent inhibitory effect on the proliferative responses stimulated with allo-antigen, Con A or PHA. IL-2 production was strongly inhibited by CsA, whereas the inhibitory activity of SASP on IL-2 production was lower than that on the proliferative response with any stimuli. Therefore, we attempted to elucidate the different mecha-

![Fig. 4. Effects of SASP and CsA on the IL-2 production stimulated with PHA. BALB/c mouse spleen cells ($5 \times 10^6$) were cultured with various concentrations of SASP (A) or CsA (B) in the presence of PHA (100 μg/ml). After 24 hr, culture supernatants were collected, and the IL-2 activity (diluted 2'-fold) was measured by the ability to induce proliferation of CTLL-2 cells. Results are means±S.D. of triplicate sets of a typical experiment in several independent studies. **: P<0.01, as compared with the respective control value.]
Effect of SASP on T cell Responses

1.5 compared with each other. SASP completely inhibited the Con A-induced proliferative response even when added 4–8 hr after initiation of culture, but, as previously reported, CsA showed only a weak inhibition when added after 4 hr or later (Table 2). Larsson and Countinho (14) demonstrated that splenocytes once activated with Con A for 4 hr can proliferate in the presence of CAS and no longer requires Con A. In this system, CsA was reported to have an inhibitory action on the initial event (20). In light of this finding, the site of action for SASP was compared with that of CsA. As shown in Table 3, Exp. 1, SASP did not inhibit the proliferative response when present only in 4 hr-pulsing with Con A, but inhibited the response when added at the later stage. On the contrary, CsA was confirmed to be effective in the early stage but not in the later stage. This mode of action of SASP and CsA was verified using purified IL-2 in place of CAS (Table 3, Exp. 2).

Discussion

Our previous study demonstrated that SASP inhibits in vitro antibody production in a T cell-dependent manner, and inhibition of anti-SRBC plaque-forming cells (PFC) production by SASP was accompanied with a reduction of IL-2 secretion (8). In this study, effects of SASP on the proliferative response and IL-2 production induced by alloantigen, Con A or PHA, in which T cells are mainly involved, was investigated and compared with those of CsA. As a result, it was found that SASP as well as CsA inhibited proliferative responses induced by three different stimuli in a dose-dependent fashion. As for IL-2 production in the above mentioned responses, CsA showed a marked inhibitory effect, whereas the inhibitory activity of SASP was weaker than that of CsA. Furthermore, SASP and CsA decreased the expression of IL-2 R induced by Con A, and the inhibitory effect was more marked in CsA. The principal mechanism of T cell function inhibition by CsA is reported to involve the inhibition of IL-2 and the IL-2 R system (9–13). On the other hand, in the case SASP, it may be difficult to explain the inhibitory effect only by inhibition of the IL-2 system. In other words, SASP appears to differ from CsA in the mechanism of
Table 2. Effects of SASP and CsA added after various times of culture on the Con A-induced proliferative response

<table>
<thead>
<tr>
<th>Time of addition (hr)</th>
<th>medium</th>
<th>SASP 2×10⁻⁴ M</th>
<th>CsA 100 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>83260±9901</td>
<td>2233±1438</td>
<td>9070±2055</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>1710±365</td>
<td>42245±2514</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>1464±279</td>
<td>43399±2140</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>1625±512</td>
<td>56677±1370</td>
</tr>
</tbody>
</table>

SASP (2×10⁻⁴ M) or CsA (100 ng/ml) was added to the cultures of Con A (5 μg/ml)-stimulated spleen cells at the indicated time. The values are means±S.D. of triplicate sets of a typical experiment in several independent studies.

Table 3. Effect of SASP and CsA on the elicitation of proliferative response by CAS or IL-2

<table>
<thead>
<tr>
<th>Addition</th>
<th>³H-TdR uptake (cpm)</th>
<th>Con A-4 hr pulse with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>medium</td>
<td>SASP</td>
</tr>
<tr>
<td>Exp. 1</td>
<td>medium</td>
<td>5142±806</td>
</tr>
<tr>
<td></td>
<td>CAS</td>
<td>77891±648</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(80)</td>
</tr>
<tr>
<td></td>
<td>CAS+SASP</td>
<td>18869±517</td>
</tr>
<tr>
<td></td>
<td>(24)</td>
<td>(14)</td>
</tr>
<tr>
<td></td>
<td>CAS+CsA</td>
<td>70231±2305</td>
</tr>
<tr>
<td></td>
<td>(90)</td>
<td>(13)</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>medium</td>
<td>2273±518</td>
</tr>
<tr>
<td></td>
<td>IL-2</td>
<td>9890±601</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(118)</td>
</tr>
<tr>
<td></td>
<td>IL-2+SASP</td>
<td>4070±337</td>
</tr>
<tr>
<td></td>
<td>(41)</td>
<td>(79)</td>
</tr>
<tr>
<td></td>
<td>IL-2+CsA</td>
<td>7789±323</td>
</tr>
</tbody>
</table>

The values are means±S.D. of triplicate sets of a typical experiment in several independent studies. Cells were stimulated with Con A (5 μg/ml) in the presence or absence of SASP (2×10⁻⁴ M) or CsA (100 ng/ml) for 4 hr. The stimulated cells were collected and incubated for 30 min in 20 mg/ml α-MM, 10 mM HEPES, 10% FCS-MEM. After washing, they were recultured with medium, SASP or CsA in the presence of 50% v/v CAS (Exp. 1) or 100 U/ml of purified human IL-2 (Exp. 2).

Inhibition of the proliferative responses.

T lymphocyte activation is caused by antigens or mitogens and promoted by soluble factors, lymphokines. Larsson et al. established the step-wise proliferative response induced by Con A (14, 21, 22). In the early step, resting T cells acquire the responsiveness to IL-2 within 4 hr of the Con A-pulse, which is independent of la⁺ accessory cells. In the latter step, these competent cells proliferate in response to IL-2, whose production is dependent on la⁺ cells. Furthermore, Larsson (20) reported that CsA (1–10 μg/ml) acts on the initial step resulting in the inhibition of the proliferative response of T cells. Bunjes et al. (9) reported that CsA exerts an inhibitory effect on induction of cytotoxic T cells when present only within the first 24 hr of culture. Moreover, Kumagai et al. (19) demonstrated that the early stage of T cell activation by phorbol esters and calcium ionophores is sensitive to CsA, which results in the inhibi-
tinction of IL-2 and IL-2 R. On the contrary, SASP showed its inhibitory effect on the Con A-
induced proliferative response by acting in the phase where activated lymphocytes pro-
liferate dependent on CAS or IL-2. The values of 3H-TdR uptake of preactivated cells in-
duced by hIL-2 alone was lower than that by CAS (Table 3). A possible explanation for this is
that IL-2 used in this experiment, 100 U/ml, may not be sufficient to maintain the prolife-
ration or that factors other than IL-2, included in CAS, may participate in the proliferation.
Sheldon et al. (23) reported that the delayed addition of SASP to the Con A-stimulated cells up to 46 hr after the onset of the culture resulted in inhibition of the response, although the degree of suppression decreased with the passage of time. We have also shown that SASP is necessary in the middle of the culture to inhibit the anti-SRBC PFC response (8). From these observations, SASP appears to act on the latter phase in the T cell response distinct from CsA. The different site of action of SASP and CSa may be due to a difference of the hydrophilicity or lipophilicity. Recent studies have revealed the existence of a specific cytosolic protein that binds to CsA, termed cyclophilin, which is responsible for the inhibitory action of CsA (24).

The mechanism for how SASP inhibits the Con A response, although still unclear, has been speculated to be as follows: The first is that SASP may directly prevent the binding of IL-2 and IL-2 R or that SASP may inhibit signal transduction at some step occurring after the binding of IL-2 to IL-2 R. The second is that SASP may strongly inhibit the production of lymphokines other than IL-2 which is involved in the T cell activation. IL-4 has been characterized as a proliferation-promoting factor for activated T cells (25) and also reported to act as a cofactor in combination with IL-2 in the polyclonal activation of resting T cells by phorbol ester (26). In addition, granulocyte-macrophage colony stimulating factor (GM-CSF) has been shown to accelerate T cell proliferation (27, 28). In addition, Bickel et al. (29) demonstrated that CsA fails to inhibit the GM-CSF production of activated T cells. Therefore, it may be of considerable interest to determine the effect of SASP on IL-4 and GM-CSF production.

When SASP is orally administered, it is partially absorbed, and the rest is cleaved by bacteria in the colon into SP and 5-ASA. 5-ASA remains in the intestine and is finally excreted in the feces, whereas SP is absorbed and further metabolized (30). The immunopharmacological activities of metabolites are still controversial. In this study, SP and 5-ASA were demonstrated to be not as effective as SASP in inhibiting the proliferation and IL-2 production in MLR. We also previously described that SASP (1×10⁻⁵-5×10⁻⁴ M) significantly suppressed the anti-SRBC PFC response, and its suppressive efficacy overwhelmed that of SP or 5-ASA (8). In agreement with our results, it was reported that only SASP has an inhibitory effect on the mitogen-induced proliferative response using lymphocytes from various species (23, 31, 32). On the contrary, Symmons et al. (33) showed that not only SASP but also SP and 5-ASA inhibit the pokeweed mitogen-in-
duced proliferation of peripheral blood mono-
nuclear cells from healthy volunteers and RA patients. As has been discussed, we still do not know the reason why inconsistent results of inhibitory activity of metabolites were obtained. Nevertheless, SP was shown to be effective in RA as reported by Pullar et al. (34) and Neumann et al. (35). This fact suggests that SP, as well as SASP, may participate in the anti-rheumatic action through its antimicrobial effect or other immunopharmacolog-
ical effects (36, 37).

References
1 Svartz, N.: The treatment of rheumatic poly-
arthrits with acid azo compounds. Rheumatism
4, 56-60 (1948)
2 Scinclair, R.J.G. and Duthie, J.J.R.: Salazopyrin
Rheum. Dis. 8, 226-231 (1949)
3 McConkey, B., Amos, R.S., Butler, E.P.,
Crockton, R.A., Crockton, A.P. and Walsh, L.: Salazopyrin in rheumatoid arthritis. Agents Ac-
tions. 8, 438-441 (1978)
4 Neumann, V.C., Grindulis, K.A., Hubball, S.,
McConkey, B. and Wright, V.: Comparison
5 Pullar, T., Hunter, J.A. and Capell, H.A.: Sulpha-
salazine in rheumatoid arthritis: a double blind


28 Woods, A., West, J., Rasmussen, R. and Bottomly, K.: Granulocyte-macrophage colony stimulating factor produced by cloned L3T4a+...
Effect of SASP on T cell Responses


