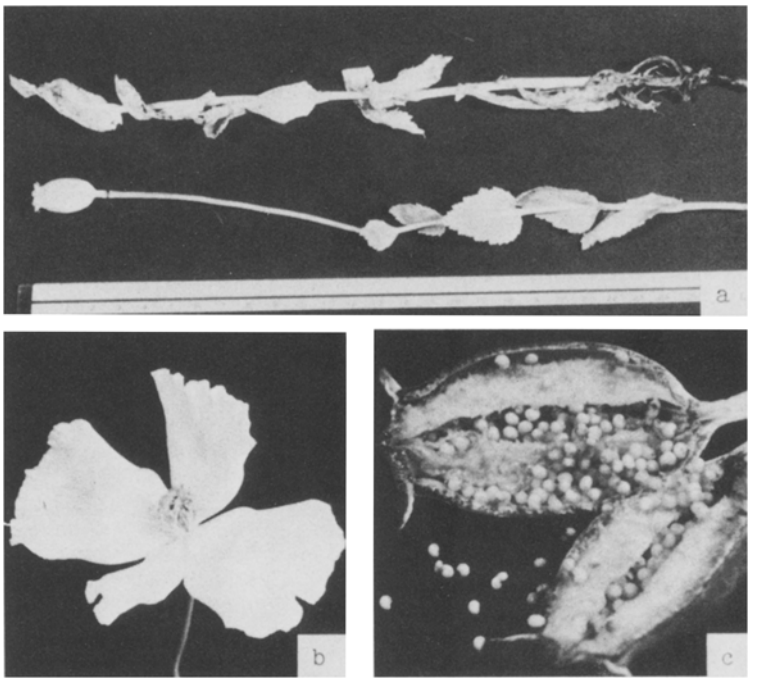

Figure 2. Plantlets developed in soil after regeneration from callus cultures of *Papaver somniferum*. *a* The plantlets cultivated for 3 months after transferring into soil in pot from clonal shoots (fig. 1,d). *b* The flower of a plantlet developed in soil from clonal shoots, which had 4 different sizes of petals. *c* The mature capsule of a plantlet developed in soil, which contained many seeds.

height and the leaves were normal and a serrate shape (fig. 1,d). Finally, the regenerated plants flowered in vitro under the optimal light conditions, 16 h photoperiod at about 7000 lx. The flower formed in vitro from the callus continued to blossom for 3 weeks (fig. 1,e), although that of the original plant grown from a seedling fell within a day. The flower generated in vitro had 4 white petals, a pistil and many anthers, but the petals were curly and the capsule, which developed fully from the pistil (fig. 1,f), did not contain any seed and latex. This fact suggests that the pollen was not formed in the anthers or remained immature.

On the other hand, the plantlets which formed roots in vitro (in the step of fig. 1,d) were transferred into soil in pots, and grew to about 50 cm in height after 3 months (fig. 2,a). The flower then produced closely resembled those of the original plant, except that one pair of petals was extremely small as compared to the other one (fig. 2,b). The fully matured capsule was about 2 cm in height and 1.5 cm in width and contained the latex and many seeds (fig. 2,c). Recently, we confirmed that the seeds had the potential to germinate. Moreover, we found that various tissues which regenerated from the callus cultures contained the morphinan alkaloids. The main alkaloid found was codeine, although the original plants produced morphine as the major alkaloid[9]. The morphinan alkaloid content of each tissue in various differentiation steps is under investigation.

1 Part 41 in the series 'Studies on plant tissue cultues'. For Part 40 see Furuya, T., Yoshikawa, T., Orihara, Y., and Oda, H., J. natl Prod. (1983) in press.
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Effect of alloimmunized thymic cells on isolated mouse atria. Participation of prostaglandins[1]

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Summary. The effects of thymic cells from alloimmunized mice on the mechanical activity of isolated mouse atria were explored. Immune cells decreased the tension without changing the rate of beating of the atrium. After inhibition of prostaglandin synthesis with indomethacin and acetylsalicylic acid, the negative inotropic action of alloimmunized thymic cells was blocked.

The heart has been shown to release in vitro a number of oxidative metabolites of arachidonic acid (AA) in response to changes in oxygen tension[3], hormone stimulation[4], nerve stimulation[5,6], and mechanical damage[7], and during cardiac anaphylaxis[8].

The synthesis of prostaglandins (PGs) and related lipids is

influenced by a variety of factors associated with immunological regulation. Also, accessory cells such as those of the monocyte-macrophage series have active cyclooxygenase without exception[9]. The synthesis of PGs and thromboxane A_2 (TXA_2) has been reported to occur in glass wool-adherent mouse splenic lymphocytes[10], peripheral blood lymphocytes[11] and mouse thymocytes[12].

The purpose of this study is to evaluate the action of thymocytes obtained from alloimmunized mice on the spontaneous activity of isolated mouse atria; a negative inotropic effect was assessed. In order to investigate the mechanism of this action, the influence of inhibitors of PG synthesis as well as antihistaminic drugs was studied.

Methods. Animals. Young adult (2-4 months) inbred BALB/c and closed colony CF1 mice from our breeding facilities were used throughout.

Immunizations. Immunizations were done with pooled lymphoid cells (from spleen, lymph nodes and thymus) obtained by pressing the organ through a stainless steal mesh. Cells were suspended in phosphate buffered isotonic solution (PBS). All immunizations were carried out between animals of the same sex. BALB/c mice were immunized by one intradermal injection of 10^7 CF1 lymphoid cells followed by 1-4 boosters of 3×10^7 CF1 lymphoid cells i.p. at weekly intervals.

Preparation of cell suspension. All animals were bled 4-6 days after the last injection and their thymus removed and pressed through a mesh using Eagle's minimal essential medium (MEM) (Gibco). After pelleting, cells were treated once with 0.75% $ClNH_4$ in 0.02 Tris buffer pH 7.2 and washed 3 times with MEM. Finally, they were resuspended in RPMI 1640 tissue culture medium with glutamine (Gibco) with 5% fetal calf serum and gentamicine (80 µg/ml) at a final concentration of $2-4 \times 10^7$ cells/ml. The viability of the cells was assessed by the trypan blue exclusion test.

Antibody titers. In order to evaluate the degree of immunization, sera were titered for cytotoxic antibodies using a trypan blue exclusion test in a 1-step complement-dependent cytotoxic assay. In short, CF1 thymus cells were incubated with 1:2 serial dilutions of the test sera; guinea-pig serum was added as a source of complement and 50% lysis was used as the titration point[13].

Atrial preparations. Mice of the CF1 strain were decapitated and their auricles removed and suspended in a modified Krebs-Ringer-Bicarbonate (KRB) solution gassed with 5% CO_2 in oxygen, maintained at pH 7.4 and 30 °C; the ionic composition of KRB was reported elsewhere[14]. After 1 h of equilibration, initial values for the isometric tension and the frequency of the spontaneously beating atria were measured, as previously described[15]. Cumulative dose-response curves for thymocytes (normal and immunized) were constructed for untreated atrial controls as well as for auricles preincubated for 30 min with indomethacin (10^{-6} M) or acetylsalicylic acid (1.8×10^{-4} M) or pyrilamine (10^{-6} M). Experimental records were compared with initial controls and expressed as percentage change. Differences

Absolute magnitude of tension of mouse auricles. Effects of indomethacin, acetylsalicylic acid and pyrilamine

Conditions	Tension (mN)[a]	n
Untreated[b] (control)	1.27±0.09	8
Pyrilamine[c] (10^{-6}M)	1.16±0.12	5
Indomethacin[c] (10^{-6}M)	1.32±0.14	5
Acetylsalicyclic acid[c] (1.8×10^{-4}M)	1.26±0.11	5

[a] Means±SEM. [b] Initial values recorded at 10 min following equilibration (see methods). [c] Values at 30 min after addition.

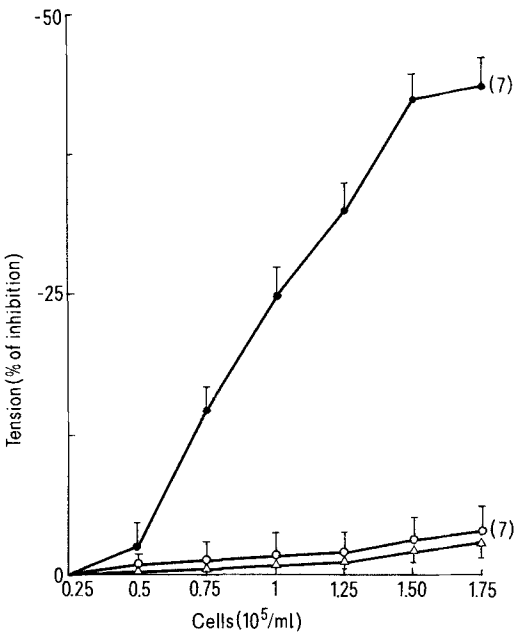

Figure 1. Cumulative dose-response curves of the inotropic action of thymocytes on isolated CF1 mouse atria. Effect of thymocyte from BALB/c immunized with CF1 (●——●), control BALB/c (○——○) and control CF1 (△——△). Changes in tension are expressed as percent of change below initial control. Points and vertical bars are mean ± SEM. Figures between parentheses indicate the number of preparations.

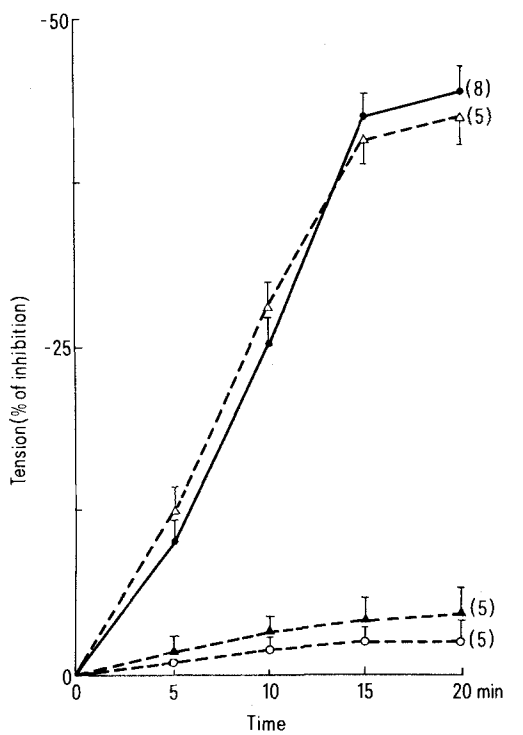

Figure 2. Time course of the reaction of immunized BALB/c thymocytes on mouse atria. Isolated atria were suspended in KRB without inhibitory agents (●——●) or with 10^{-6} M indomethacin (▲---▲); 1.8×10^{-4} M acetylsalicylic acid (○---○) or 10^{-6} M pyrilamine (△---△). 1.5×10^5 thymocytes/ml were added. Other details as in figure 1.

between mean values were considered significant if $p = 0.05$ or less.

Results. As shown in figure 1, the addition of thymocytes from BALB/c mice that had received 4 immunizations of CF1 cells to isolated CF1 mouse atria beating in KRB, strongly decreased the tension without modifying the frequency (not shown in figure) of the beating atrium. The degree of inhibitory action was directly proportional to the concentration of the immune effector cells in the medium. On the contrary, control thymocytes from BALB/c or CF1 mice had no significant effect on isolated CF1 mice atria. Furthermore, BALB/c anti CF1 thymocytes had no effect on isolated rat atria (not shown). It is interesting to note that the effect of immune thymocytes could be recorded after the 2nd injection and did not increase with further immunizations; in the mean time, the cytotoxic titers of these same animals went from 1/8 (2 immunizations) to 1/128 (5 immunizations).

As may be seen in figure 2, the in vitro inhibitory effect induced by alloimmunized thymocytes developed with time and was maximal after 15 min. Figure 2 also shows a significant inhibition of the negative inotropic effects of alloimmunized thymocytes, when atrial preparations were preincubated during 30 min with indomethacin or aspirin. Pyrilamine did not have any visible effect. It should be noted that the tension of control atria, or those treated with indomethacin, acetylsalicylic acid and pyrilamine had similar absolute values before the addition of experimental cells (table).

Discussion. This study demonstrates that thymus cells from alloimmunized mice can react in vitro with spontaneously-beating mouse atria, decreasing the contractile tension in a concentration-dependent manner, but not affecting the frequency of contractions. The action of thymocytes was very rapid and could be observed shortly after exposure of the atria to the effector cells. On the other hand, normal BALB/c or CF1 thymocytes were ineffective in this system. This points to the necessity of preimmunization to bring about the response.

The fact that the negative inotropic action was not modified by antihistaminic drugs suggests that the release of histamine was not involved in this effect. On the contrary, the inhibition of the cyclooxygenase activity with indomethacin or acetylsalicylic acid[16] antagonized the action of immune cells. This would indicate that the active substance producing the negative inotropic action could be derived from arachidonic acid via the cyclooxygenase-catalyzed pathway. Although it is recognized that all the blocking drugs used do not have specific actions, it is possible to suggest that during the reaction of isolated mouse atria with alloimmune thymocytes, active substances derived from the cyclooxygenase-catalyzed pathway are generated.

It has previously been shown that only PGs E series have negative inotropic action without changes to the rate of contraction[15] while PGI_2, PGF_2alpha and TXB_2[17-20] stimulate both tension and frequency of atria.

The question whether or not thymocytes synthesize prostaglandins and related lipids is a matter of controversy. It has been reported[12] that mouse thymus cells contain and synthesize the PGE series. Mature thymocytes contain a much higher concentration of PGE and have an increased activity of prostaglandin synthetase compared with immature cells. It is accepted that thymocytes exhibit many functions of peripheral T lymphocytes, such as mitogen responsiveness and graft vs host reactivity[21,22]. Available data demonstrate that type E prostaglandins modify various immune responses[23] inducing the expression of certain thymocyte differentiation antigens, but it has to be demonstrated that it is accompanied by change in function.

In the experimental model presented in this study it is not yet possible to know whether the cyclooxygenase product(s) that mediate the reaction are released at the effector cell surface or whether they are the results of the activation of the enzymes of the heart tissue upon interaction with alloimmunized thymocytes. That the interaction exists, is supported by the fact that normal thymocytes do not exert any action; while immunized thymocytes do. It is proposed that on recognition of alloantigens expressed by atrial cells, thymocytes are activated and release either PGE or some factors that induce this release by other cells. It is not known yet whether cell-to-cell contact is necessary or whether soluble mediators (lymphokines) are involved. Nevertheless, this work demonstrates the capacity of alloimmunized thymocytes to trigger biological effects in myocardial tissue.

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