中国药理学报 *Acta Pharmacologica Sinica* 1989 May; **10** (3): 270-274

Effect of insulin on the production of interleukin 2 by T lymphocytes

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Abstract The effect of insulin on interleukin 2 (IL-2) production was investigated. The results showed that insulin alone did not stimulate the resting T lymphocytes to produce IL-2, but enhanced the production of IL-2 by T lymphocytes and interleukin 1 (IL-1) by macrophages in the presence of lectin. Insulin augmented the response of T lymphocytes to IL-1 in the presence of Con A. The peak time of IL-2 production was not affected by insulin.

Key words insulin; interleukin 2; interleukin 1; T lymphocytes; macrophages

Insulin, a polypeptide hormone, plays an important role in immune regulation, such as enhancement of lymphocyte activation[1,2], cytotoxic response of T cells[3] and mixed lymphocyte cultures (MLC) reaction *in vitro*[4] etc. As so far, it was not known how insulin regulates lymphokines. In this paper, we present evidence that insulin enhances IL-2 production in the presence of lectin and the mechanism for regulation of insulin on T lymphocyte activation is analyzed.

Materials and methods

Human peripheral blood The peripheral blood of healthy volunteers was obtained from Wuhan Blood Centre. The blood was defibrinated usiug sterile glass beads.

Mice C 57 BL/6 mice, 6 to 8-wk-old (for obtaining peritoneal macrophages) and 3 to 4-wk-old (for obtaining thymocytes), were purchased from Experimental Animal Centre of Tongji Medical University.

Insulin Crystaline insulin (purchased from Shanghai Chemical Reagent Store) was dissolved in RPMI 1640 (pH 7.0) and

Received 1988 Apr 16 Accepted 1988 Dec 3

stored for no longer than 1 wk at 4℃.

Preparation of human IL-2 Human peripheral blood lymphocytes (HPBL) were isolated from defibrinated blood by Ficoll-Hypaque density centrifugation[1]. The cells were suspended at a cell density of 2×10^6 cells /ml in RPMI 1640 supplemented with 1% human serum and purified phytohemagglutinin (PHA-P, Difco) 10 μg/ml in absence or presence of insulin, and then cultured in 24-well culture plates (Costar) at 37℃, in 5% CO_2 with a humid atmosphere. After 40 to 44 h, supernatants were collected by centrifugation ($600\times g$ for 20 min) and stored at −20℃ until assay for IL-2 activity. The supernatants were designated as human peripheral blood lymphocyte conditioned medium (HPBL-CM).

Assay for IL-2 activity T lymphoblasts were isolated from HPBL stimulated with PHA-P for 4 d by Ficoll-Hypaque density centrifugation, and cultured in 96-well culture plate (Costar) at a cell density of 2×10^4 cells/well in absence or presence of HPBL-CM. After 64 h, cell cultures were pulsed with [^{3}H]TdR (0.018 MBq/well) for 8 h. The cells were harvested onto glass fiber filters with cell harvester, and [^{3}H]TdR incorporation was determined by liquid scintillation counting. The results were expressed as the means of cpm of [^{3}H]TdR in triplicate cultures.

Preparation of mouse IL-1 Mouse IL-1 was prepared as reported earlier[5]. Briefly, peritoneal macrophages from 6 to 8-wk-old mice were cultured at a cell density of 1×10^6 cells/ml in RPMI 1640 supplemented with 10 μg/ml lipopolysaccharide (LPS, Sigma) and 10% new born calf serum (NBCS) in the presence or absence of insulin in 96-well culture plates. After 48 h, the supernatants were collected by centrifugation and stored at −20℃ until assay for IL-1 activity. The supernatants were disignated as Mϕ-CM.

Assay for IL-1 activity Thymocytes from 3 to 4-wk-old mice were treated with nylon wool to remove the adherent cells[5] and cultured in 96-well culture plate (5×10^5 cells/well) in RPMI 1640 supplemented with 10% NBCS in the presence of 5 μg/ml Con A and Mϕ-CM. After 56 h, thymocyte cultures were pulsed with 0.018 MBq of [^{3}H]TdR for 16 h. The cells were harvested onto glass fiber filters and [^{3}H]TdR incorporation was determined as above.

Results

Effect of insulin on IL-2 production The ability of IL-2 production by T cells stimulated with PHA and/or insulin was tested. The insulin preexsisted in HPBL-CM wouldn't influence the result of assay of IL-2 activity, because insulin is inactive after incubated at 37℃ for 40 h and cryopreserved at −20℃ (data not shown). As show in table 1, insulin did not trigger the resting T lymphocytes to produce IL-2 if PHA was absent, but enhanced IL-2 production by T cells in the presence of PHA. The action of insulin to enhance IL-2 production was augmented with dose of insulin increased (Fig 1).

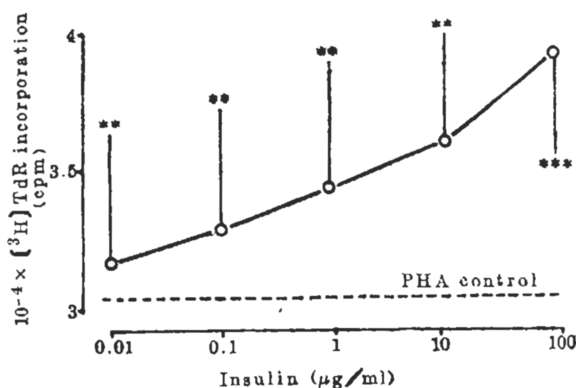

Fig 1 **Effect of insulin on IL-2 production by T lymphocytes in the presence of PHA 10 μg/ml. n = 7, $\bar{x}\pm$SD. $^{**}P<0.05$, $^{***}P<0.01$ *vs* PHA control**

Tab 1. Effect of insulin on IL-2 production. n = 5, $\bar{x} \pm SD$. *** $P<0.01$ *vs* PHA 10 μg/ml.

Phytohemagglutinin (μg/ml)	Insulin (μg/ml)	[³H]TdR uptake (cpm)
0	0	1540±301
0	10	1654±473
10	0	26671±9951
10	10	33225±8238***

It was reported[1,2] that if resting T cells was exposed to mitogen for 12 h, insulin replaced mitogen to maintain the proliferative response after removal of mitogen. To investigate the mechanism of insulin action, the following experiment was performed. HPBL was cultured in RPMI 1640 medium with PHA-P 10 μg/ml and 10% human serum for 12 h, then washed three times to remove PHA and recultured in RPMI 1640 medium with 10% human serum and 10 μg/ml insulin. The culture supernatants were collected after 36 h and 60 h, respectively, and IL-2 activity was assayed (Tab 2). Insulin replaced mitogen to maintain the coutinuation of IL-2 production by lymphocytes after removal of PHA. IL-2 activity of insulin group was higher than that of control group.

Tab 2. Effect of insulin on IL-2 production after removal of PHA. n = 4, $\bar{x} \pm SD$. ** $P<0.05$, *** $P<0.01$ *vs* control.

Initial PHA (μg/ml)	Added agents after removal of PHA: Insulin (μg/ml)	Added agents after removal of PHA: PHA (μg/ml)	[³H]TdR uptake (cpm) 36 h	[³H]TdR uptake (cpm) 60 h
10	0	0	2525±797	2934±413
10	10	0	8250±1991**	6186±265**
10	0	2.5	15731±4275***	11745±3713***

Effect of insulin on peak hours of IL-2 production HPBL was stimulated by PHA in the presence or absence of insulin, and the culture supernatants were collected at different times. IL-2 activity in supernatants was assayed. Fig 2 shows that the kinetics of IL-2 production in the presence of insulin was the same as that in the absence of insulin, but IL-2 activity of insulin group was higher than that of control group at different times, indicating that insulin enhanced IL-2 production, but did not change the peak time of IL-2 production.

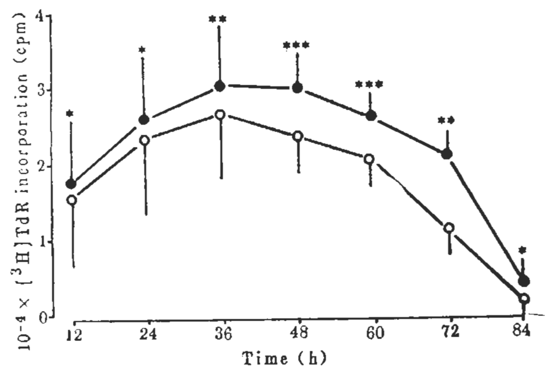

Fig 2. Kinetics of IL-2 production in the presence (●) or absence (○) of insulin. n = 4, $\bar{x} \pm SD$. * $P>0.05$, ** $P<0.05$, *** $P<0.01$ *vs* without insulin.

Effect of insulin on production and action of IL-1 Peritoneal macrophages from mice were stimulated with LPS for 48 h in the presence or absence of insulin, and the IL-1 activity in the supernatants was assayed. Insulin was added in assay system to observe the effect of insulin on the action of IL-1. Table 3 showed that [³H]TdR uptake by thymocytes in insulin

Tab 3. Effect of insulin on IL-1 production by macrophage and response of thymocyte to IL-1. n = 11, $\bar{x} \pm SD$. *** $P<0.01$ *vs* control group.

Condition in preparation	Condition in assay: Con A (μg/ml)	Condition in assay: Insulin (μg/ml)	[³H]TdR uptake (cpm)
Control	5	0	8959±4144
LPS	5	10	14446±1853***
LPS + insulin (10 μg/ml)	5	0	15081±3441***

groups (no matter whether insulin was added in preparation or in assay) was significantly higher than that of control group, indicating that insulin augmented the production of IL-1 by macrophages and enhance the response of thymocytes to IL-1.

Discussion

In previous study, insulin was not found to be mitogen of lymphocytes[1,2], but enhanced the activation of T lymphocytes in the presence of mitongen. We found that IL-2 activity in the culture supernatants did not be detected if the resting T lymphocytes were stimulated with insulin alone for 48 h. The possible reason for the result could be due to the lack of specific receptor for insulin on the resting lymphocyte surface[6]. Once the resting lymphocytes stimulated by mitogen expressed receptors for insulin, insulin regulated metabolism of T cells through an initial interaction with a specific surface receptor and enhanced IL-2 production, even if mitogen was removed. Although insulin enhanced IL-2 production, but it did not change the peak time of IL-2 production. These results indicate that insulin is a regulating factor but not a factor triggering IL-2 synthesis by T lymphocytes.

Our experiment results demonstrated that thymocytes proliferated in the presence of exogenous IL-1 after stimulation by Con A, even if adherent cells was removed by the treatment with nylon wool. Under these conditions insulin increased [^{3}H]TdR uptake by thymocytes, suggesting that insulin augmented response of thymocytes to IL-1.

IL-1 is required for the production of IL-2[7]. In this paper, we demonstrated that insulin enhanced IL-1 production by macrophages. The results obtained suggest that the effect of insulin on IL-2 production must be multiplicity, which included the effect of insulin on IL-1 production and the direct action of insulin on T cells. Enhancement by insulin of IL-2 production can be one of the mechanisms that insulin enhances the activation of T cells.

Data from experiments in which insulin regulated immune function *in vitro* led us to propose that *in vivo* hyposecretion of insulin must result in the decline of T lymphocyte-mediated immune function, which is a possible reason for immune abnomalities in diabetic animal[8,9]. The administration of insulin is surely to alleviate metabolic state as well as to strengthen the capacity of immune functions of diabetes.

[1] The research was supported by the Funds of National Natural Science.

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胰岛素对T淋巴细胞产生白细胞介素 2 的影响

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提要 胰岛素(10 μg/ml)本身不能刺激静止的 T 细胞产生白细胞介素 2(IL-2)，但在丝裂原存在时，胰岛素能促进 T 细胞产生 IL-2，而不影响 IL-2 产生的高峰时间.实验还证明：胰岛素能促进巨噬细胞产生白细胞介素1(IL-1)和增强胸腺细胞对IL-1的应答.因此胰岛素可能通过对 T 细胞代谢的直接调节和增加巨噬细胞 IL-1 的分泌来促进 T 细胞 IL-2 的产生.

关键词 胰岛素；白细胞介素1；白细胞介素2；T 淋巴细胞；巨噬细胞

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