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☐ **Brief Communication** ☐

Inhibition of entry of *Toxoplasma gondii* into MDCK cells by fetal bovine serum

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Abstract: We experienced the partial inhibition of entry of *Toxoplasma gondii* into MDCK cells when the FBS was depleted from media. MDCK cells and *Toxoplasma* (RH strain) were co-cultured, the penetration was inhibited up to 60-80% with concentration-dependence of FBS. Inhibitory effect was clear when the conc. of FBS was over 1% (v/v) with 50% inhibition conc. of 5%. When *Toxoplasma* was pre-incubated with FBS and then applied to MDCK cells, there were no inhibitory effect, but when FBS was added to *Toxoplasma*-MDCK co-culture, the time of adding was critical with rapid inhibition. And when FBS was further treated with heat (95°C, 10 min), the inhibitory effect was decreased slightly in both raw and inactivated FBS. The FBS factor(s) might participate to neutralize secreted materials which enhancing penetration or intervene between receptor-ligand binding at the moment of entry through sterically rather than functionally.

Key words: *Toxoplasma gondii*, entry, inhibition, FBS, MDCK cells

Toxoplasma gondii is an opportunistic intracellular parasite that, in the tachyzoite stage in the life cycle, is capable of infecting a wide range of nucleated cells of vertebrate hosts (Werk, 1985). *Toxoplasma* is an extraordinarily successful parasite not only in immune compromised but also in immune competent patients (Ambroise-Thomas & Pelloux, 1993). Interest in *Toxoplasma* has recently increased due to serious infections in congenitally infected foetuses, after transplant surgery and in patients with AIDS (Buxton, 1990). Despite several decades of studies on the entry mechanism of *Toxoplasma* into host cells, this theme remains interesting and there is still much scope for controversy and speculation. Based on well-known earlier observations, the penetration itself can be divided essentially into four stages: 1) recognition, 2) attachment, 3) entry, and 4) modification of the parasitophorous vacuole. We experienced the partial inhibition of penetration of *Toxoplasma* into MDCK cells *in vitro* when the fetal bovine serum (FBS) is depleted from media. We report here the inhibitory patterns of FBS on the entry of *Toxoplasma* into MDCK cells with some treatments applied to FBS.

Virulent tachyzoites of RH strain of *Toxoplasma* passaged in peritoneum of ICR mice were tested on the penetration ability into MDCK cells (ATCC CCL 34) maintained in Earle's MEM (EMEM, Gibco Laboratories) supplemented with or without FBS. Two batches of FBS were tested, that of FBS 100-106, Gemini Bioproducts Inc. and FBS 200-6140, Gibco Laboratories. MDCK cells of unsaturated density (3×10^5 cells/ml) and *Toxoplasma* (1×10^7 tachyzoites/ml) were co-cultured for 1 hour. The number of *Toxoplasma* penetrated was counted in 6 to 10 fields under the light microscope of ×400 after staining with Giemsa solution and then

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expressed by the number of *Toxoplasma* per 100 host cells. Data were obtained after 3 times experiments with triplicate each and expressed by percent inhibition which calculated with the formula: [1 − (No. of *Toxoplasma* per host cells treated/No. of *Toxoplasma* per host cells untreated)] × 100.

When the FBS of various concentrations were treated, the penetration of *Toxoplasma* into MDCK cells was inhibited up to 60-80% with concentration-dependence of FBS in spite of a little degree of differences between raw and heat-inactivated (56°C, 30 min) in two batches used. Inhibition was clear when the conc. of FBS was over 1% (v/v) with 50% inhibition conc. of 5% (Fig. 1). When *Toxoplasma* was pre-incubated with a heat-inactivated batch of FBS and then applied to MDCK cells, there were no changes in the number of *Toxoplasma* penetrated despite the duration of incubation from 5 to 60 min. But when FBS was added to *Toxoplasma*-MDCK co-culture, the time of adding was critical to the penetration of *Toxoplasma* with a pattern of rapid inhibition from 5 to 20 min then slow inhibition thereafter (Fig. 2). And one batch of FBS was further treated with heat (95°C, 10 min) whether the FBS inhibitory factor(s) may be heat stable or not. Further heat decreased the inhibitory effect in both raw and inactivated FBS, the decreasing rate was more significant in the case of raw FBS than inactivated FBS (Fig. 3).

We demonstrate here the FBS factor(s) which inhibits the penetration of *Toxoplasma* into host cells. The FBS factor(s) is fuctional not more than 1% (v/v) of media and has a characteristic of heat-labile to some degree. Since contact of host cells with the lateral or posterior part does not result in penetration, recognition must depend on appropriate orientation. Pre-incubation of *Toxoplasma* with FBS did not show the inhibitory effect, FBS factor(s) did not seem to involve in this stage. Contact with the apical part allows the involvement of the organelle of the apical complex in the penetration (Nichols & O'Connor, 1981). Physical contact readily triggers the rhoptries to extrude their contents to secrete materials enhancing penetration (Perkins, 1992) or draw out ligand to join receptor of the host cell of which the receptor-ligand were not yet identified (Nam *et al.*, 1990). At this point the FBS factor(s) might participate to neutralize materials secreted or

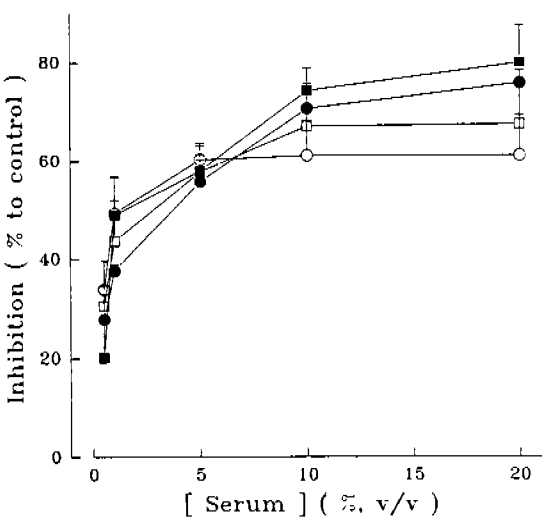

Fig. 1. Percent inhibition to control of the number of *Toxoplasma* penetrated into MDCK cells by various concentrations of FBS. ○: FBS 100-106 of Gemini Bioproducts Inc. ●: heat-inactivated (56°C, 30 min) of FBS 100-106. □: FBS 200-6140 of Gibco Laboratories ■: heat-inactivated (56°C, 30 min) of FBS 200-6140

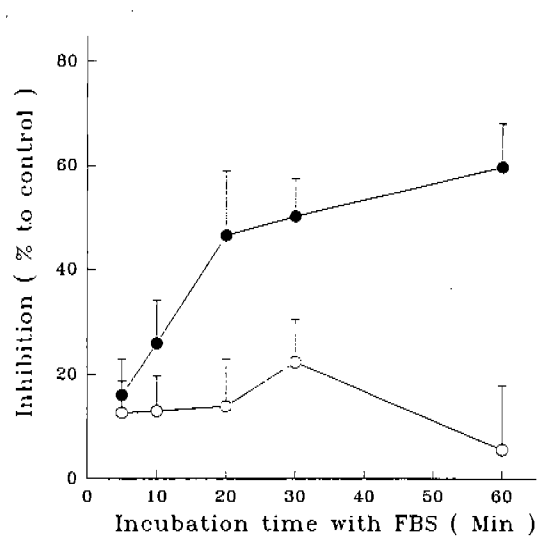

Fig. 2. Percent inhibition to control of the number of *Toxoplasma* penetrated into MDCK cells by various duration of incubation with FBS. ○: *Toxoplasma* only pre-treated with FBS ●: *Toxoplasma*-MDCK co-culture treated with FBS

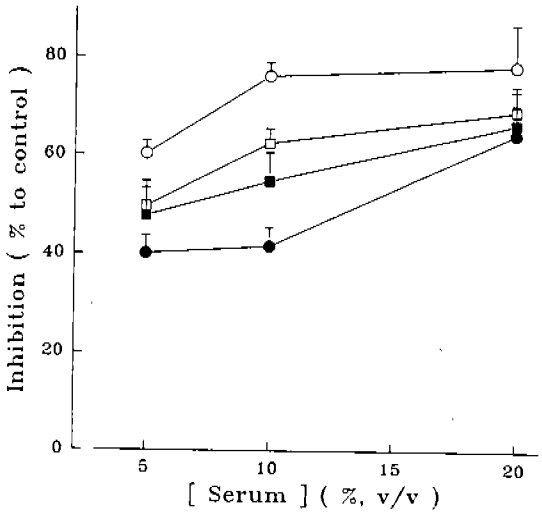

Fig. 3. Percent inhibition to control of the number of *Toxoplasma* penetrated into MDCK cells by various concentrations of FBS treated with heat at 95°C for 10 min (heat 95). ○: raw FBS ●: heat 95 treated FBS □: heat-inactivated FBS ■: heat 95 treated heat-inactivated FBS

intervene between receptor-ligand binding through sterically rather than functionally.

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=국문초록=

*Toxoplasma gondii*의 숙주세포 침투를 억제하는 우태아혈청 성분

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우태아혈청의 성분이 *Toxoplasma*의 숙주세포 침투를 억제하는 현상을 보여 이를 보고하고자 한다. MDCK 세포를 숙주로 하여 *Toxoplasma*의 RH주를 첨가하였을 때, 우태아혈청 농도 1%(v/v)에서 억제 효과가 나타나기 시작하여 5%일때 침투능을 반감시켰다. 우태아혈청의 비동화 여부는 억제 효과에 영향을 미치지 못하였으며, 우태아혈청을 95°C에서 10분간 처리하여 억제물질의 기능을 파괴시킬 때 억제 효과는 약간 감소되었다. *Toxoplasma*를 먼저 우태아혈청으로 처리한 후 숙주세포에 첨가하였을 때 침투능에는 효과를 미치지 못하였으나, *Toxoplasma*를 숙주세포와 배양하면서 우태아혈청을 첨가하였을 때에는 시간의 경과에 따라 침투능을 억제시켰다. 이상의 결과로 우태아혈청에 *Toxoplasma*의 숙주세포 침투를 억제하는 성분이 존재하는 것을 확인하였으며, 억제 방법이 침투하는 순간에 일어나며, 억제물질의 기능을 통해서라기 보다는 구조를 통해서 이루어진다고 추정할 수 있었다.

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