# ● Epidermal Growth Factor가 림프구 기능조절 작용에 미치는 영향에 관한 실험적 연구

우건희·신형식 원광대학교 치과대학 치주과학교실

림프계 세포에 대한 EGF의 작용 및 그 기전을 규명하고, EGF가 림프구기능에 미치는 영향을 시험관내에서 직접 밝히고자 시도한 연구로서 사람말초혈액 림프구를 대상으로 EGF가 이들 세포의 증식활성, cytokine과 Ig 생산능 등에 미치는 영향과 그 작용기전을 알아보기 위하여 실험하였던 바 다음과 같은 성적을 얻었다.

- 1. EGF는 PWM자극 단핵세포의 중식을 EGF를 세포-mitogen자극과 동시에 처리하면 현저히 억제시켰으나, EGF를 mitogen자극 24시간에 가하면 오히려 항진시켰다.
- 2. 세포에의 EGF 전처리는 세포의 mitogen자극 증식반응에 영향을 미치지 않았다.
- 3. 순수분리한 CD 4<sup>+</sup>세포 및 B림프구롤 PWM으로 자극배양시 EGF를 배양초에 가하면 그 증식능이 다소 억제되었으나 배양 24시간에 가하면 오히려 상승되었다.
- 4. CD  $8^+$ 세포에 의한 세포증식억제활성은 CD  $8^+$ 세포를 CD  $4^+$ 세포와 단핵구를 혼합배양시에만 발현되었으며, EGF는 CD  $8^+$ +CD  $4^+$ +단핵구혼합배양에 의한 CD  $8^+$ 의 억제활성을 현저히 항진 시켰다.
- 5. CD 8<sup>+</sup>억제활성은 IL1, IL2, IFN-γ가 공존시는 더욱 항진되었다.
- 6. EGF에 의한 CD 8<sup>\*</sup>세포의 억제활성항진은 FGE₂ 및 IFN-γ분비촉진과 밀접한 관계를 보였다.
- 7. EGF는 단핵구의 IL1분비 및 CD  $4^+$ 세포의 IL2와 IFN- $\gamma$ 분비를 촉진시켰으나 B림프구의 IL6분비는 억제시켰다.
- 8. SAC자극 B세포를 IL2존재하에 배양시 EGF를 SAC자극과 동시에 처리하면 증식능 및 IgG생산능이 모두 반감되었으나 EGF를 SAC자극 24시간에 처리하면 증식능 및 IgG생산능 모두 영향을 받지 않았다. 그러나 SAC자극 B세포를 IL6 존재하에 배양시 IgG생산능은 EGF처리에 의하여 현저히 항진되었으며 그 항진의 정도는 EGF를 SAC자극 후에 처리시 더욱 뚜렷하였다.
- 9. EGF는 고밀도 B세포의 IgG생산능에는 영향을 미치지 않았으나, 저밀도세포의 IgG생산능은 IL6 존재하에서 현저히 항진되었다.
- 10.EGF는 IL2에 대한 표적세포의 감응도에는 영향을 미치지 못하였으나, IL6에 대한 표적세포의 반응은 현저히 항진시켰다.
- 이상의 결과로 EGF의 림프구기능 조절작용은 세포주기 및 시간의존성이며, EGF가 이들 세포에 직, 간접으로 작용하여 복잡한 기전으로 발현됨을 알 수 있었다.

### ● 치은 섬유아세포 배양시 합성되는 단백질에 관한 연구

이관훈·정진형 단국대학교 치과대학 치주과학교실

세포의 성장과 분화는 혈청 및 여러 성장인자들에 의해 영향을 받으며 이에 대해 최근 많은 연구가되고 있다. 본 실험은 치은 섬유아세포 성장인자와 혈청의 영향을 알아보고자 정상 치은조직으로부터

- 1. Fusobacterium nucleatum (n=48) was highly susceptible to penicillin G and polymixin B, but not to metronidazole.
- 2. Eikenella corrodens (n=48) was highly susceptible to minocycline and augmentin, but low susceptable to metronidazole, phosphomycin, polymyxin B.
- 3. Wolinella recta (n=48) was highly susceptible to minocycline and augmentin, but resistant to phosphomycin, metronidazole, polymyxin B.
- 4. Porphyromonas gingivalis (n=8) was highly susceptible to almost antibiotics, especially to ampicillin, minocycline and augmentin, but resistant to metronidazole.
- 5. Prevotella intermedius (n=36) was highly susceptible to minocycline, augmentin and cephalothin, but very resistant to phosphomycin, metronidazole, polymyxin B and Cephalosporin C.
- 6. Actinobacillus actinomycetemcomitans (n=48) was susceptible to ampicillin, penicillin G and cephalothin, but very resistant to phosphomycin, metronidazole and polymyxin B.
- 7. All the bacterial species tested was highly susceptible to penicillin G, augmentin, ampicillin comparatively, but resistant to metronidazole, phosphomycin, polymyxin B and cephalosporin C.
- 8. In β-lactamase assay, only one species of Wolinella recta showed the production of β-lactamase.

As mentioned above, in results of the experiment for proper antibiotic agent selection, as supplemental therapeutic agents for rapidly progressive periodontitis of Korean, penicillin G, ampicillin, augmentin, minocycline and doxycycline are considerably effective for regression of anaerobic bacterial growth in dental plaque.

In future, confronted problem of above antibiotics in that development of program for own-effect-exhibiting method in gingival sulcus and proper plan for resistant groups are necessary.

## In vitro effect of epidermal growth factor on the regulation of lymphocyte function

#### Gun Hee Woo, Hyung Shik Shin

Dept. of Periodontology, School of Dentistry, Won Kwang University

Epidermal growth factor(EGF), single-chain polypeptid(MW 6045) has been known as potent extracellular regulator of biological responses in a wide variety of cell types. However, little has been reported about the role of EGF in the regulation of lymphocyte-function. Moreover, the mechanisms of EGF-action on immune system has not yet been elucidated.

The purpose of this study was to investigate on the in vitro effect of EGF on proliferation of T and B lymphocytes, the production of cytokines and Ig, and some mechanisms responsible for its regulating effect on lymphocyte-functions.

Monocytes, CD 4<sup>+</sup>, CD 8<sup>+</sup> and lymphocytes was isolated from the pheripheral mononucleus cell of healthy person, their proliferation, differentiation and the production of cytokine and Ig by EGF was measured. Also, the effect of cell, prostaglandin E<sub>2</sub>, IFN-γ and some interleukin(IL) on the proliferation, differentiation and the production of cytokine and Ig by EGF measured. Also, the effect of

cell, prostaglandin  $E_2(PGE_2)$  IFN- $\gamma$  and some interleukin(IL) on the proliferation and differentiation of mononucleus cell was measured by using sensitive cell such as CTLL 2, mouse thymocytes, B9 cell, Wish cell.

- 1. When EGF was added to culture during the programming stage of activation, EGF inhibited the proliferative responses of peripheral blood mononuclear cells significantly.
- 2. The responses were somewhat augmented if EGF was added to culture after mitogen activation, and were not influenced if EGF was added before activation.
- 3. The poke weed mitogen(PWM)-induced proliferative responses of purified CD 4<sup>+</sup> and B lymphocytes were slightly decreased when EGF was added at 24hr after mitogen-activation.
- 4. The modulating effect of EGF on lymphocyte proliferation was time and dose dependent, and the potent suppression was manifested only when CD 4<sup>+</sup> cells and B lymphocytes were cocultured with monocytes and CD 8<sup>+</sup> cells.
- 5. The differentiation of CD 8<sup>+</sup> surppressor cells in PWM-stimulated cultures required soluble factors elaborated by CD 4<sup>+</sup> cells and monocytes.
- 6. EGF did not influence the differentiation of CD 8<sup>+</sup> cells directly, and EGF-induced augmentation of CD 8<sup>+</sup> cell-differentiation was developed through enhanced cytokine production of monocytes and CD 4<sup>+</sup> celss.
- 7. The monocyte-signal for such differentiation could be replaced not by IL1 but by  $FGE_2$  and the CD  $4^+$  cell-signal, not by IL2 but by interferon gamma.
- 8. By exposure of monocytes, IL2 and IFM-γ production of CD 4<sup>+</sup>cells were significantly enhanced, but IL6 production of Staphylococcus aureus Cowan 1(SAC)-activated B cells was markedly down-regulated. IgG secretion of SAC-activated B cells was remarkably decreased by adding EGF to culture at initiation.
- 9. In exogeneous IL6 was added to cultures, Ig-secretion of EGF-B cells was significantly increased than that of EGF-free control. When fractionated B cells were cultured in the presence of exogeneous IL6, IgG secretion of high-density small B cells was not influenced by EGF, but Ig secretion of low density large cells in EGF-group was significantly enhance than that of control.
- 10. EGF did not alter the IL2-dependent CTLL2 cell-responsiveness to IL2, but it did greatly increase the responsiveness of target(B9) cells to IL6 with about two-fold increment.

These results suggested that EGF has multiple effects on event controlling lymphocyte-functions in a time-dependent manner via modulation of releasing soulble factors.

### The study on the protein synthesized by cultured human gingival fibroblast

#### Kwan Hoon Lee, Chin Hyung Chung

Dept, of Periodontology, school of Dentistry, Dankook University

This experiment was performed to study the effects of fibroblast growth factor(FGF) and fetal bovine serum(FBS) on the protein synthesis and proliferation of human gingival fibroblasts.

Gingival tissues obtained from healthy human were diced into 1mm³ and cultured in α-MEM contai-