Article No. ck970328

CHEMOTAXIS AND CHEMOTACTIC SELECTION INDUCED WITH CYTOKINES (IL-8, RANTES AND TNF-α) IN THE UNICELLULAR TETRAHYMENA PYRIFORMIS

László Kőhidai and György Csaba

Three representative cytokines interleukin (IL-8), RANTES and tumour necrosis factor α (TNF-α) have a concentration-dependent chemotactic effect on the unicellular Tetrahymena. Maximal effective concentrations of IL-8 (1 ng/ml) and RANTES (75 ng/ml) are in the same range as in mammals, which indicates an evolutionary background of physiological effects elicited. Progeny generations of cells selected for their affinity to cytokines (IL-8 and TNF-α) show an enhanced positive chemosensory reaction to the cytokines. The changed reaction of these cells to the chemoattraction of the culturing medium was also observed. The results call attention to the presence of cytokine-dependent processes at a low phylogenetic level.

Chemotactic responses belong to the most basic physiological activities of both unicellular and multicellular organisms; however, with different roles at different phylogenetic levels. In the early stages of evolution, short peptides, carbohydrates and other nutrients are thought to have been responsible as ligands for inducing chemotactic responses. In the consecutive steps of signalling development, other more complex ligands, receptors or receptor-like structures, and signal pathways appeared.[1] Unicellular organisms of the present day respond to a scale of peptides[2,3] or steroids[4] as signal molecules via receptor-mediated mechanisms.[5]

In higher phylogenetic levels chemoattractant and chemorepellent substances are present in a wide range of tissues with different physiological and pathological activities, participating in fertilization,[6] angiogenesis,[7] or vascular and extravascular migration during inflammation.[8] The specificity of these different responses are well determined and tuned by a network formed between the tissue/cell-released soluble substances, e.g. chemokines, and the target, migratory cells. In mammals, among others, three cytokines—IL-8, RANTES and TNF-α—possess characteristic, well-studied chemotactic potential.[9–11] The network of signalling by cytokines is complex and, although they have common targets of action, their molecular structure differs (C–C and C–X–C chemokines, or other cytokines).[12] Their producers also vary: IL-8 is released predominantly by monocytes,[13] macrophages,[14] fibroblasts[15] and endothelial cells;[16] RANTES is synthesised by eosinophils,[17] fibroblast-like synoviocytes,[18] platelets[19] and macrophages;[20] and TNF-α is a characteristic product of monocytes, macrophages[21] and mast cells.[22] The effector cells of these cytokines/chemokines are also diverse: IL-8 promotes chemotaxis of neutrophils,[23] RANTES acts on macrophages,[24] eosinophils[25] and T cells,[24] while TNF-α induces the migration of fibroblasts.[26] These facts indicate that reactions to chemokines are important in both basic and clinical research, however we have no data about their chemotactic effects at a low eukaryotic phylogenetic level.

Tetrahymena, a ciliated protozoan, is frequently used as a model cell in cellular and molecular biology and cell physiology.[27] The suitability of this ciliate as a model is based on its similarity to the mammals in respect to membrane structure and function;[28] the presence of significant second messenger pathways: cAMP,[29] cGMP,[30] IP_3,[31] Ca^{2+}-calmodulin;[32] and inducibility of basic physiological responses like growth,[33] phagocytosis,[34] metabolism,[35] etc. In addition, chemosensitivity is one of the most essential physiological responses of these unicellular organisms. Previous studies have proved that a large group of signal molecules, including hormones,[36] lectins[37] and short peptides,[38] have the capacity to induce specific

From the Department of Genetics, Cell- and Immunobiology, Semmelweis University of Medicine, Budapest, Hungary
Correspondence to: György Csaba, Department of Genetics, Cell and Immunobiology, Semmelweis University of Medicine, 1089 Budapest, Nagyvárad tér 4, Hungary
Received 22 April 1997; revised and accepted for publication 26 November 1997

1043–4666/98/070480 + 06 $30.00/0

KEY WORDS: chemotaxis/ cytokine/ selection/ Tetrahymena

chemotactic responses. Some of the substances tested, like the bacterial origin tripeptide fNle–Leu–Phe, which has a strong chemoattractant effect on neutrophils,[39] and its antagonist variant, suggested evolutionary conclusions and the suitability of using this protozoa as a model cell of chemotaxis.

In the present experiments, two basic problems were studied: (1) To test whether, after application of the three cytokines, IL-8, RANTES and TNF-α, these molecules have any potency to elicit chemosensitive responses in a Tetrahymena model. (2) To characterize this probable chemotactic response from a new perspective, namely "chemotactic selection". This way of evaluation provides information about positively responding cells and their chemotactic responses in offspring generations. In this way, we could characterize the drive/potency of the three cytokines to select our model cells, and also the homogeneity of the selected populations to the given cytokine.

RESULTS

Concentration course of chemotaxis

There was a diverse chemotactic response induced by the three cytokines. Concentration course analysis (Fig. 1) demonstrated that IL-8 has chemotactic potency on Tetrahymena with maximal, most significant effect at low, 1 ng/ml concentration, however 0.5 ng/ml concentration also induced chemotaxis. In the higher concentration range there was a distinct peak at 25 ng/ml with a mild chemotactic potency, but 20 and 50 ng/ml concentrations of IL-8 had strong chemorepellent effects. RANTES also promoted chemotactic behaviour, but only at the high concentrations, with maximal effect at 75 ng/ml. Below this concentration this substance could not induce chemotaxis of the unicellular model cell. The third cytokine, TNF-α, had only one detectable significant chemotactic peak at 1 ng/ml. Lower and higher concentrations elicited chemo-repellent effects, underlined both in the high (10 ng/ml) and low range (0.1–0.5 ng/ml) of concentrations.

Chemotactic selection

IL-8 showed a significant chemotactic effect in group Control/IL-8 (Fig. 2). The chemotactic responses of IL-8-selected groups were diverse up to the substance applied the second time. Cells of the IL-8/Control group demonstrated a strong chemotactic response towards the control substance itself. However, IL-8, the substance applied for selection, could induce a significantly higher response.

Potency to select cells by chemotaxis was partly different in TNF-α groups. Cells selected by control medium (Control/TNF) showed enhanced chemotactic

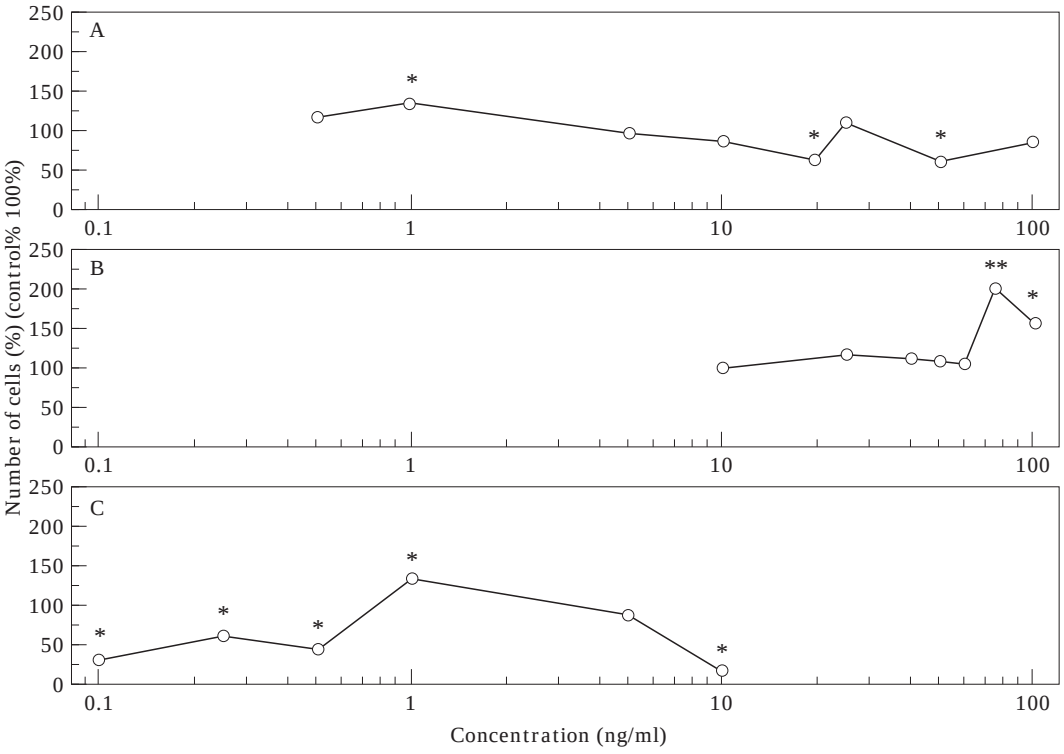

Figure 1. Concentration course of the chemotactic effect elicited by three cytokines in Tetrahymena.

*$P < 0.01$; †$P < 0.001$. A: IL-8; B: RANTES; C: TNF-α.

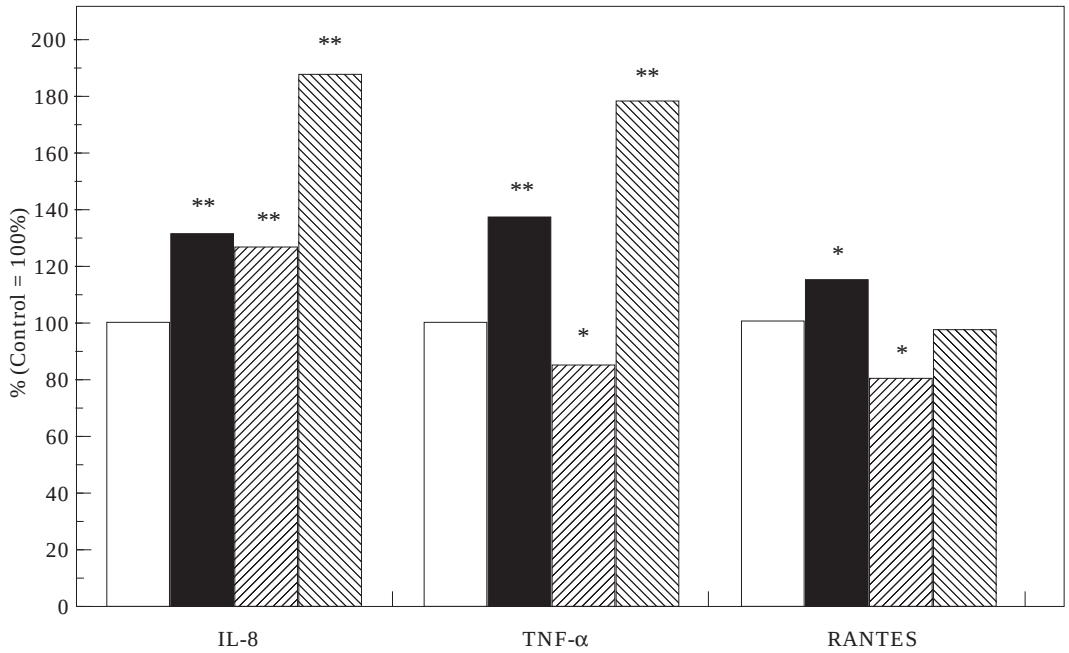

Figure 2. Chemotactic response to fresh medium and to the identical cytokine in populations selected by "chemotactic selection" induced by three cytokines.

IL-8 (concentration 1 ng/ml), TNF-α (concentration 1 ng/ml) and RANTES (concentration 75 ng/ml). (□), Control/Control; (■), Control/Cytokine; (▨), Cytokine/Control; (▧), Cytokine/Cytokine. $*P < 0.05$; $**P < 0.01$.

behaviour, identical to that of the control cells of the first part of the experiment, when TNF-α was applied the second time (Fig. 2). Chemotactic activity of subpopulations selected with TNF-α varied according to the substance applied the second time. These cells did not prefer control medium, which had a mild repellent effect on TNF/Control cells. In contrast, the TNF-α elicited very significant chemotactic responses in the TNF-selected group (TNF/TNF).

Effects of RANTES were different from the two cytokines mentioned above (Fig. 2). RANTES could work as an attractant on the cells selected with control media (Control/RANTES). The subpopulations selected with RANTES had negative or low responsiveness at the second time, as the control media could elicit significant chemo-repellent behaviour (RANTES/Control) and RANTES itself did not induce any chemotactic response (RANTES/RANTES).

DISCUSSION

Selection seems to be a crucial step of chemotactic processes in higher organisms. During inflammation, formation of the leukocyte subsets and their directional migration is highly regulated by several substances, such as products of bacteria as well as the complement cascade, leukotriene metabolites secreted by different cells of the micro-environment, and chemokines the compounds tested here.[40–42] Chemokines—members of the superfamily of cytokines—have the potency to induce selective, non-random locomotion of different cells at diverse tissue loci: primary neutrophil rolling and adhesion, followed by emigration of cells through the wall of postcapillary venules, and, finally, the initiation of effector, phagocytic events. Selective chemokine signals promote the accumulation of monocytes, T and B lymphocytes at the site of inflammation. Consecutively, products of these cells are multipotent, as they initiate respiratory burst (in monocytes), degranulation and the release of several enzymes, among which there are also direct and indirect chemotactic factors.[43]

Members of the chemokine family have high (20–45%) homology. They are classified into two main subgroups: C–C and C–X–C chemokines. Grouping is based on sequence homology, particularly the content of a conserved structural motif: in C–C group there is a pair of cysteines close to the amino-terminal end of the molecule, while in the members of C–X–C group there is an amino acid inserted between the two cysteines. Chemokine receptors responsible for signal transduction also demonstrate high identity, e.g. receptor of the C–X–C molecule IL-8 and receptors of C–C chemokines demonstrate 32% identity.[44]

The chemokines tested in this experiment represent these two groups: RANTES belongs to C–C chemokines, while IL-8 is a member of the C–X–C chemokine subfamily.[12] The third molecule, TNF-α, although not a member of chemokines, is a key

molecule as this substrate is one of the most essential factors released by cells recruited during inflammation and it works as a strong inducer of IL-8 synthesis.[45]

On the basis of the literature mentioned above, we can evaluate our results as molecule-dependent chemotactic responses elicited by basically different peptides possessing chemotactic character.

Concentration course analysis of chemokines (IL-8 and RANTES) indicates that unicellular Tetrahymena has similar receptorial and efferent mechanisms, concerning specific chemotactic response, to those of higher, multicellular organisms. Comparison of the most effective concentrations of these two molecules shows that the sensitivity of Tetrahymena to both substances is in the same range as in mammals.[24,46] However, in contrast to mammals, Tetrahymena exhibits higher sensitivity to IL-8 (1 ng/ml vs 4 ng/ml), while exhibiting lower sensitivity to RANTES (75 ng/ml vs 50 ng/ml). The one-peak chemoattractant effect of TNF-α indicates the specificity of this molecule at a low concentration (about 1 ng/ml) and its repellent effect or toxicity at lower or higher concentrations, respectively.

In the "selection experiments" a population was selected and sustained of cells, which were sensitive to the "invitation" of cytokines at the first encounter. The progeny generations of these cells were studied after 1 week (about the 70th generation) for chemosensory response to the culturing medium alone, or to this medium and the cytokine provoking the selection. By this arrangement of the experiments we could evaluate the selecting capacity of a chemotactic signal, which could have had, an important role during the evolution of signalling systems. The use of the culturing medium as control in the selection experiments was necessary, as its chemoattractant effect was known from our previous reports.[2,36]

The study of selection indicated the different potentials of cytokines to select cells. The three cytokines had positive chemotactic potency at the first encounter. The chemotactic response of the offspring generations was different. The response of the cells selected with two cytokines (RANTES and TNF-α) was decreased to the medium (RANTES/C and TNF/C), which shows that the sensitivity of these populations is changed compared to the control, while IL-8 had no such effect. The specificity of cells selected was further studied when the same cytokine was applied at the second time as well. Selection with the C–X–C chemokine IL-8 and TNF-α had the potential to "collect" cells possessing higher responsiveness than the mixed cultures. We might interpret this enhanced chemotactic response as a sign of selection, on the basis of receptor mediated mechanisms. Though the presence of IL-8 receptor has not been detected, the positive chemotactic activity of fMet–Leu–Phe to Tetrahymena[38] and the 77% homology found in the receptors of the tripeptide and IL-8[47] also indicate the specificity of the response elicited. In addition to the mechanisms discussed above, there is a possibility that cytokines at the first selection provoked an imprinting-like effect[1] on the model cells. Although we can not exclude the possibility of this mechanism, according to our previous experiments, 15-min treatment was not enough to develop changes detectable by binding studies.[48]

In the case of the C–C chemokine RANTES the chemotactic selection of cells was not a long lasting one. We cannot presently explain this fact, however, the chemotactic profile of RANTES was uncertain (see Fig. 1), considering the fluctuating behaviour of the concentration curve.

In conclusion, Tetrahymena proved to be a good model to evaluate selective chemotaxis elicited by cytokines/chemokines. Our results, in accord with previous data from the literature, demonstrate that these signal molecules have more background in evolution than was considered before.[27,49] The experiments call attention to the diverse reaction of Tetrahymena to different cytokines, manifested in the different chemosensory activity in the case of the first encounter and in the different selectioning capacity of cytokines.

MATERIALS AND METHODS

Cells and culturing

Cultures of Tetrahymena pyriformis GL were used in the logarithmic phase of growth. The cells were sustained in a medium containing 1% tryptone (Difco, Michigan, USA) and 0.1% yeast extract (Difco) at 28°C.

Chemotaxis assay

Measurement of chemotactic activity was carried out according to Leick[50] in a test modified by us.[51] The time of incubation was 15 min. After incubation, the contents of the inner chamber were removed and cells were fixed in 4% formaldehyde containing PBS.

The samples were measured using a Neubauer cytometer. Each experiment was repeated five times.

In the first part of the experiments the concentration courses were evaluated. The chemokines were obtained from: human recombinant IL-8 (Promega, Madison, WI); human recombinant RANTES (Promega, Madison, WI); and TNF-α (Sigma Chemical Co., St Louis, MO). The following groups and concentrations were tested: (1) Control—fresh culture media was used as "attractant"; (2) IL-8—0.5, 1.0, 5, 10, 20, 25, 50, 100 ng/ml; RANTES—10, 25, 40, 50, 60, 75, 100 ng/ml; TNF-α—0.1, 0.25, 0.5, 1.0, 5, 10 ng/ml.

Substances were diluted in fresh culture medium immediately before the experiments. The concentrations of substances applied were chosen according to the data

provided by the literature testing higher organisms for physiological responses.[52–54]

Chemotactic selection

In the second part of the experiment "chemotactic selection" was carried out. Set-up of the chemotaxis assay was similar to the first part. However, these experiments were done under sterile air-flow. The concentrations of the three chemokines for these assays were chosen according to their most effective concentration of the concentration course.

In this set-up, cells of the outer chamber were considered as "mixed population", and the drive of chemotactic substances, filled into the inner chamber, were applied to select populations on the basis of their chemotactic preference. In control groups fresh culture medium was applied as chemoattractant. After each run, selected cells (of the inner chamber) were transferred into fresh culture media. The cultures formed this way were transferred every third day. After a week, cultures were assayed again. The following groups were formed (the first word indicates the type of selection/the second indicates the chemoattractant applied one week later):

IL-8—Control/Control, Control/IL-8, IL-8/Control, IL-8/IL-8;
RANTES–Control/Control, Control/RANTES, RANTES/Control, RANTES/RANTES;
TNF-α—Control/Control, Control/TNF, TNF/Control, TNF/TNF.

Counting of samples was done as in the basic experiment.

Statistical evaluation

For statistical evaluation, Origin 2.8 and Statistica were used. These provided the values of Student's *t*-test, standard deviation and variance.

Acknowledgements

This work was supported by the National Research Fund (OTKA T-013355 and T-017773) Hungary. The two chemokines interleukin-8 (IL-8) and RANTES were generous gifts of Promega (Madison, WI, USA).

REFERENCES

1. Csaba G (1980) Phylogeny and ontogeny of hormone receptors : the selection theory of receptor formation and hormonal imprinting. Biol Rev 55:47–63.

2. Csaba G, Kőhidai L (1995) Effects of L-alanine and L-alanine peptides on the chemotaxis of Tetrahymena: evolutionary conclusions. Biosci Rep 15:185–190.

3. Kőhidai L, Csaba G (1995) Effects of the mammalian vasoconstrictor peptide, endothelin-1, on Tetrahymena pyriformis GL, and the immunological detection of endogenous endothelin-like activity. Comp Biochem Physiol C 111:311–316.

4. Csaba G, Inczefi-Gonda Á, Fehér T (1985) Induction of steroid binding sites (receptors) and presence of steroid hormones in the unicellular Tetrahymena pyriformis. Comp Biochem Physiol A 82:567–570.

5. Christopher GK, Sundermann AC (1995) Isolation and partial characterization of the insulin binding sites of Tetrahymena pyriformis. Biochem Biophys Res Commun 212:515–523.

6. Ralt D, Manor M, Cohen-Dayag A, Tur-Kaspa I, Ben-Shlomo I, Makler A (1994) Chemotaxis and chemokinesis of human spermatozoa to follicular factors. Biol Reprod 50:774–785.

7. Koch AE, Polverini PJ, Kunkel SL, Harlow LA, DiPietro LA, Elner VM, Elner SG, Strieter RM (1992) Interleukin-8 as a macrophage-derived mediator of angiogenesis. Science 258:1798–1801.

8. Mibu Y, Shimokaway Y, Hayashi H (1985) Lymphocyte chemotaxis in inflammation. X. Heterogeneity of chemotactic responsiveness in human T subsets towards lymphocyte chemotactic factors from delayed hypersensitivity reaction site. Immunology 55:473–479.

9. Rajarathnam K, Sykes BD, Kay CM, Dewald B, Geiser T, Baggiolini M, Clark-Lewis I (1994) Neutrophil activation by monomeric interleukin-8. Science 264:90–92.

10. Taub DD, Lloyd AR, Wang JM, Oppenheim JJ, Kelvin DJ (1993) The effects of human recombinant MIP-1 alpha, MIP-1 beta, and RANTES on the chemotaxis and adhesion of T cell subsets. Adv Exp Med Biol 351:139–146.

11. Postlethwaite AE, Seyer JM (1990) Stimulation of fibroblast chemotaxis by human recombinant tumor necrosis factor alpha (TNF-alpha) and a synthetic TNF-alpha 31-68 peptide. J Exp Med 172:1749–1756.

12. Baggiolini M, Dewald B, Moser B (1994) Interleukin 8 and related chemotactic cytokines CXC and CC chemokines. Adv Immunol 55:97–179.

13. Teranishi Y, Mizutani H, Murata M, Shimizu M, Matsushima K (1995) Increased spontaneous production of IL-8 in peripheral blood monocytes from the psoriatic patient: relation to focal infection and response to treatments. J Dermetol Sci 10:8–15.

14. Apostolopoulos J, Davenport P, Tipping PG (1996) Interleukin-8 production by macrophages from atheromatous plaques. Arterioscler Thromb Vasc Biol 16:1007–1012.

15. Lonnemann G, Engler-Blum G, Muller GA, Koch KM, Dinarello CA (1995) Cytokines in human renal interstitial fibrosis. II. Intrinsic interleukin (IL)-1 synthesis and IL-1-dependent production of IL-6 and IL-8 by cultured kidney fibroblasts. Kidney Int 47:845–854.

16. Berger SP, Seelen MA, Hiemstra PS, Gerritsma JS, Heemskerk E, van-der-Woude FJ, Daha MR (1996) Proteinase 3, the major autoantigen of Wegeners granulomatosis, enhances IL-8 production by endothelial cells in vitro. J Am Soc Nephrol 7:694–701.

17. Ying S, Meng Q, Taborda-Barata L, Corrigan CJ, Barkans J, Assoufi B, Moqbel R, Durham SR, Kay AB (1996) Human eosinophils express messenger RNA encoding RANTES and release biologically active RANTES protein. Eur J Immunol 26:70–76.

18. Mehindate K, al-Daccak R, Schall TJ, Mourad W (1994) Induction of chemokine gene expression by major histocompatibility complex class II ligands in human fibroblast-like synoviocytes. Differential regulation by interleukin-4 and dexamethasone. J Biol Chem 269:32063–32069.

19. Kameyoshi Y, Dorschner A, Mallet AI, Christophers E, Schroder JM (1992) Cytokine RANTES released by thrombin-stimulated platelets is a potent attractant for human eosinophils. J Exp Med 176:587–592.

20. Devergne O, Marfaing-Koka A, Schall TJ, Leger-Ravet MB, Sadick M, Peuchmaur M, Crevon MC, Kim KJ, Schall TT, Kim T (1994) Production of the RANTES chemokine in delayed-type hypersensitivity reactions: involvement of macrophages and endothelial cells. J Exp Med 179:1689–1694.

21. Cui X, Zhang R, Fu W, Cao Z (1995) Pentoxiphylline ameliorates pulmonary damage caused by Streptococcus pneumoniae infection in mouse. Chin Med J Engl 108:864–869.

22. Malaviya R, Ikeda T, Ross E, Abraham SN (1996) Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha. Nature 381:77–80.

23. Hammond ME, Lapointe GR, Feucht PH, Hilt S, Gallegos CA, Gordon CA (1995) IL-8 induces neutrophil chemotaxis predominantly via type 1 IL-8 receptors. J Immunol 155:1428–1433.

24. Schall TJ, Bacon K, Toy KJ, Goeddel DV (1990) Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. Nature 347:669–671.

25. Rot A, Krieger M, Brunner T, Bischoff SC, Schall TJ, Dahinde CA (1992) RANTES and macrophage inflammatory protein 1 alpha induce the migration and activation of normal eosinophil granulocytes. J Exp Med 176:1489–1495.

26. Smart SJ, Casale TB (1994) Pulmonary epithelial cells facilitate TNF-apha-induced neutrophil chemotaxis. A role for cytokine networking. J Immunol 152:4087–4094.

27. Csaba G (1985) The unicellular Tetrahymena as a model cell for receptor research. Int Rev Cytol 95:327–377.

28. Thompson Jr GA, Nozawa, Y (1977) Tetrahymena: A system for studying dynamic membrane alterations within the eukaryotic cell. Biochim Biophys Acta 472:55–92.

29. Schultz JE, Schonborn C (1994) Cyclic AMP formation in Tetrahymena pyriformis is controlled by a $K^{(+)}$-conductance. FEBS Lett 356:322–326.

30. Kőhidai L, Barsony J, Roth J, Marx SJ (1992) Rapid effects of insulin on cyclic GMP location in an intact protozoan. Experientia 48:476–481.

31. Kovács P, Csaba G (1987) The role of Ca^{2+} in hormonal imprinting of the Tetrahymena. Acta Physiol Hung 69:167–179.

32. Nagao S, Banno Y, Nozawa Y, Sobue K, Yamazaki R, Kakiuchi S (1981) Subcellular distribution of calmodulin and calmodulin-binding sites in Tetrahymena pyriformis. J Biochem (Tokyo) 90:897–899.

33. Wheatley DN, Rasmussen L, Tiedtke A (1994) Tetrahymena: a model for growth, cell cycle and nutritional studies, with biotechnological potential. Bioessays 16:367–372.

34. Nilsson JR (1979) Phagotrophy in Tetrahymena. In Levandowsky M, Hunter SH, Provasoli L (eds) Biochemistry and physiology of protozoa, Academic Press, New York, pp. 339–381.

35. Hill DL (1972) The biochemistry and physiology of Tetrahymena. Academic Press, New York.

36. Kőhidai L, Karsa J, Csaba G (1994) Effects of hormones on the chemotaxis in Tetrahymena—Investigations on receptor memory. Microbios 77:75–85.

37. Kőhidai L, Csaba G (1996) Different and selective chemotactic responses of Tetrahymena pyriformis to two families of signal molecules: lectins and peptide hormones. Acta Microbiol Immunol Hung 43:83–91.

38. Leick V (1992) Chemotactic properties, cellular binding and uptake of peptides and peptide derivatives: studies with Tetrahymena thermophila. J Cell Sci 103:565–570.

39. Kőhidai L, Kovács P, Csaba G (1994) Chemotactic response of unicellular Tetrahymena to a leukocyte attractant peptide and its repellent derivate. Evolutionary conclusions. Cell Biol Int Rep 18:119–122.

40. Dahinden C, Galanos C, Fehr J (1983) Granulocyte activation by endotoxin. I. Correlation between adherence and other granulocyte functions, and role of endotoxin structure on biologic activity. J Immunol 130:857–862.

41. Krauss AH, Nieves AL, Spada CS, Woodward DF (1994) Determination of leukotriene effects on human neutrophil chemotaxis in vitro by differential assessment of cell motility and polarity. J Leukoc Biol 55:201–208.

42. Cavaillon JM (1995) Les cytokines de l'inflammation. CR Seances Soc Biol Fil 189:531–544.

43. Jinquan T, Frydenberg J, Mukaida N, Bonde J, Larsen CG, Matsushima K, Thestrup-Pedersen K (1995) Recombinant human growth-regulated oncogene-alpha induces T lymphocyte chemotaxis. A process regulated via IL-8 receptors by IFN-gamma, TNF-alpha, IL-4, IL-10, and IL-13. J Immunol 155:5359–5368.

44. Neote K, DiGregorio D, Mak JY, Horuk R, Schall TJ (1993) Molecular cloning, functional expression, and signaling characteristics of a C-C chemokine receptor. Cell 72:415–425.

45. Mukaida N, Matsushima K (1992) Regulation of IL-8 production and the characteristics of the receptors for IL-8. Cytokines 4:41–53.

46. Capsoni F, Minonzio F, Ongari AM, Zanussi C (1989) A new simplified single-filter assay for "in vitro" evaluation of chemotaxis of chromium-51-labelled polymorphonuclear leukocytes. J Immunol Methods 120:125–132.

47. Murphy PM, Tiffany HL (1991) Cloning of complementary DNA encoding a functional human interleukin-8 receptor. Science 253:1280–1283.

48. Kőhidai L, Csaba G (1990) Impact of the length of exposure to peptides of different molecular mass on the establishment of imprinting in Tetrahymena. Acta Protozool 29:315–319.

49. Csaba G, Kovács P, Falus A (1995) Human cytokines interleukin (IL)-3 and IL-6 affect the growth and insulin binding of the unicellular organism Tetrahymena. Cytokine 8:771–774.

50. Leick V, Helle J (1983) A quantitative assay for ciliate chemotaxis. Anal Biochem 135:466–469.

51. Kőhidai L, Lemberkovics É, Csaba G (1995) Molecule dependent chemotactic responses of Tetrahymena pyriformis elicited by volatile oils. Acta Protozool 34:181–185.

52. Ahuja SK, Murphy PM (1996) The CXC chemokines growth-regulated oncogene (gro) alpha, GRO beta, GRO gamma, neutrophil-activating peptide-2, and epithelial cell-derived neutrophil-activating peptide-78 are potent agonists for the type B, but not the type A, human interleukin-8 receptor. J Biol Chem 271:20545–20550.

53. Bischoff SC, Krieger M, Brunner T, Dahinden CA (1992) Monocyte chemotactic protein 1 is a potent activator of human basophils. J Exp Med 175:1271–1275.

54. Mathias S, Dressler KA, Kolesnick RN (1991) Characterization of a ceramide-activated protein kinase: stimulation by tumor necrosis factor alpha. Proc Natl Acad Sci USA 88:10009–10013.