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Basic fibroblast growth factor improves cellular immunological functions in mice

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Abstract

Basic fibroblast growth factor (bFGF, FGF-2) is a multifunctional growth factor that has been proved to have important roles in various tissues. Background researches revealed that biological functions of B and T lymphocytes producing bFGF and its receptors were regulated by the cytokine, which implied that bFGF may participate in immune defense. In order to investigate the effects of bFGF on immune functions, the present paper employed mice administrated with bFGF to detect phagocytosis of macrophages, activity of natural killer cell and number of antibody-producing cells in vivo as well as proliferation of spleen cells in vitro. Results showed that the four detected parameters were all significantly upregulated in bFGF-treated mice, which suggested an active role of bFGF occurring in cellular immunity of mice. Moreover, the increment of plaques formed by antibody-producing cells implied that bFGF also has a positive effect upon humoral immunity.

Keywords: Basic fibroblast growth factor (bFGF), Cellular immunity, Mice.

Introduction

Basic fibroblast growth factor (bFGF, FGF-2) is a member of FGF family comprising of more than twenty members.[1] Background researches revealed that this growth factor has pleiotropic roles in different cell types and tissues. Biological roles of bFGF are performed through two pathways: in one way, bFGF produced in cytoplasm is translocated to nucleus and ribosome to regulate gene expression; in the other way, bFGF secreted through autocrine or paracrine pathway binds to high-affinity receptors located in extracellular matrix or membrance to perform biological roles through mitogen-activated protein kinase (MAP-kinase) and phospholipase C (PLC) pathways.[2-4]

Up to date, a large number of reports have showed main effects of bFGF on development and differentiation, such as mesoderm induction, angiogenesis, vessel wall, lung, hematopoiesis, nervous system, reproductive system, skin, eye, muscle, skeleton, digestive system, and so on.[2] Some researchers found that macrophage and T lymphocyte produced bFGF,[5-8] Shen and co-workers speculated that bFGF may, directly or indirectly, play a role in T lymphocytes transformation,[9] which indicated that bFGF may be involved in immunity. However the direct evidences about the immunological functions of bFGF are still undiscovered. To confirm the speculation this paper determined the cellular immunity in bFGF-exposed mice.

Materials and Methods

Erythrocyte collection of chicken and sheep

Blood was sampled 1 ml in a tube containing anticoagulation agent under sterile conditions from jugular vein of sheep or wing vein of chicken respectively. Samples were centrifuged at 1000 rpm for 15min at 4℃. The sediments were harvested, washed three times with normal saline and then stored in 4℃ before use.

Alexin preparation

Blood of a guinea pig was isolated from heart. Serum was separated by standing at 4℃, collected and stored at 4℃ for use.

Basic FGF administration

Male Kunming mice (body weight of 18-20g) were allocated randomly into three groups. Intraperitoneal injection method was used for administration of bFGF for all animals. One group of mice were injected with bovine recombinant bFGF (br-bFGF) preserved in our laboratory[10] at 0.5 μg per mouse, another group mice at 1.0 μg per mouse and the third group as vehicle control injected with normal saline. Injection was operated every three days for three successive times.

Plaque forming cell

Plaque forming cell was used to detect the number of antibody-producing cells.[11] Sheep red blood cells were suspended to density of 1×108 cells/ml in normal saline and injected into abdominal cavity of mouse at 1 ml per animal. After five days, spleens were collected and made into cell suspension. Surface medium mixed with Hanks' solution (0.5 ml), sheep blood cell (50 μl , 1:10 dilution in phosphate buffer) and spleen cell suspension (10 μl) were added together into a eppendorf tube, mixed quickly and dumped onto cell glass slide over-spread with thin agar gel. Following solidification of the medium, the glass slide was incubated at 37℃, 5% CO_2 in a humidified atmosphere for 1.5 h. Afterwards, alexin (1:5 in phosphate buffer) was added into slot of the slide shelf and number of plaques was counted after incubation for another 1.5 h.

Phagocytosis test of celiac macrophage

Detection of phagocytosis of macrophages was performed as described.[12] Briefly, bFGF-exposed mice were used to celiac injection of 0.5 ml 1% starch. After three days, 2 ml of normal saline was also injected into cavum abdominis, abdomen was massaged for 3 min, celiac fluid was collected and transferred to an axenic eppendorf tube and centrifuged at 1000 rpm for 15 min. Sediment was harvested and re-suspended with DMEM/F-12 supplemented with 10% fetal bovine serum (FBS) to 2×106 cells/ml. The cell suspension was inoculated into 96-well plate at 100 μl per well and incubated at 37℃ 5% CO_2 in a humidified atmosphere for 4 h. After medium replacement, 1×105 chicken red cells were added into each well and incubated for 4 h. Then, numbers of phagocytized chicken red cell, total macrophage and macrophage that have phagocytized chicken red cells were counted respectively. Phagocytosis percentage (PP) and phagocytosis index (PI) were calculated as follows:

PP= (number of macrophage that has phagocytized chicken red cell / number of total macrophage) ×100%

PI= number of phagocytized chicken red cell / number of total macrophage

Detection of cells proliferation from spleen

Proliferation of spleen cell was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolum bromide(MTT) assay. Murine spleen was collected from the mouse untreated with bFGF or normal saline under sterile conditions and made into cell suspension that was diluted to 5×106 cells per ml with DMEM/F12 containing 10% FBS, bFGF and concanavalin A (10 μg/ml). Cells were seeded into 96 well-plates at 100μl per well, and cultured at 37℃ 5% CO_2 in a humidified atmosphere for 48 h. 20 μl of MTT solution in concentration of 5mg per ml was added into wells. After incubation for 6 h, each well was added with 100 μl buffer containing 10% sodium dodecyl sulfate and 10% Triton X-100 dissolved in HCl-isopropanol. The plate was vibrated for 20 min and incubated for 4 h. At the end, optical density values were read in enzyme-linked immunosorbent assay instrument at wavelength 595 nm, reference wavelength was 655 nm.

Detection of natural killer cell activity

To detect activity of natural killer cells, a previously described method was followed.[13] Murine spleen was made into cell suspension under sterile conditions and incubated in DMEM/F12 containing 10% FBS at 37℃, 5% CO_2 in a humidified atmosphere for 2 h to discard adhesive cells. Suspended cells were diluted to density of 2×106 cells per ml with medium; meantime, the K-562 cell suspension was prepared at density of 1×105 cells per ml with medium. 90 μl K-562 cell suspension, 90 μl effector

cell suspensions and 10 μl MTT were added in turn into 96-well cell culture plate and incubated for 4 h. Control wells were only added with effector cells or K-562 cells. The plate was centrifuged at 1500 rpm at 4℃ for 15min and supernatant was discarded. The plate was added with dimethyl sulfoxide 150 μl per well and vibrated for 5 min on a vibrator. Optical density value was read in enzyme-linked immunosorbent assay instrument at wavelength 570nm. Natural killer activity was calculated as follows:

Activity of natural killer = 1— (optical density value of experimental well — optical density value of control well/ optical density value of K562) ×100%

Data and statistical analysis

Results are expressed as mean ± SEM. Statistical analysis was performed using SPSS software. A probability values (P) lower than 0.05 was set to indicate the significant differences statistically.

Results

Phagocytosis percentage and phagocytosis index

Macrophage is a multifunctional immunocyte and an important object subjected to investigate cytophagy, cellular immunity and molecular immunity. Since it is easy to harvest, culture and purify, the immunocyte is frequently selected for studying body's immune functions. Phagocytosis percentage and phagocytosis index are two direct parameters reflecting immune function of macrophage. This paper detected the two parameters and showed the data in figure 1. The results indicated that among the three groups, phagocytosis percentage and phagocytosis index were detectable in macrophages after culture, and that the two parameters in the two experimental mice were significantly higher than that in control mice.

Plaque forming cells

Plaque forming cell test is a detection technology used to identify the cells that can secret antibodies against red blood cells, the measurement technique is frequently employed to detect immunology indexes that reflect the effects of some drugs on immune functions. In this paper, plaque forming cell test was used in assessing level of antibody produced by antibody-producing cells or/and quantity of anti-producing cells. The Figure 2 showed us that under combinational actions of antibody and alexin, sheep red cells underwent hemolytic reaction and resulted in plaque forming in agar gel. In our results, the numbers of plaques in the two bFGF-administered groups were significantly higher than that in the control group.

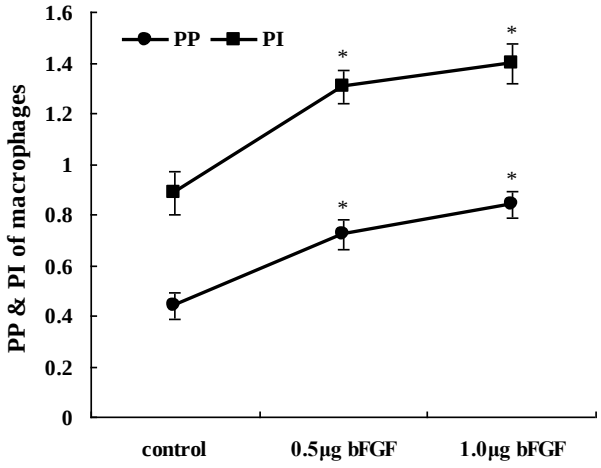

Figure 1: Effects of bFGF on macrophages. PP and PI of macrophages in two experimental groups were both higher than those in control group, *P<0.05.

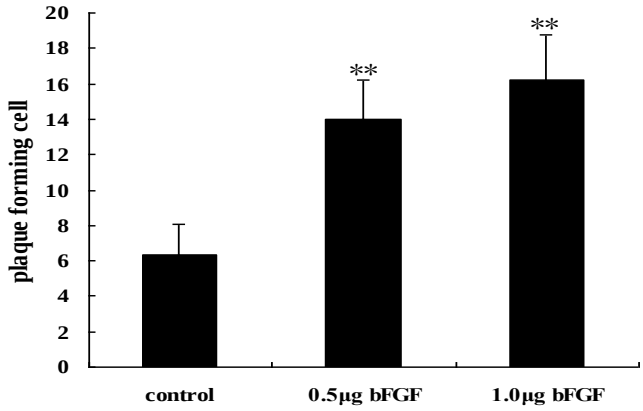

Figure 2: Effects of bFGF on antibody producing cells. Results showed that number of plaque in 0.5μg and 1.0 μg bFGF groups are higher than that of control group, **P<0.01, significantly.

Activity of natural killer cells

Natural killer cell is an important immunocyte that is not only connected with antitumour, antivirus and immunomodulation, also participates in development of hypersensitivity and autoimmune disease under certain circumstances. Therefore activity of the cell is manifestation of immune conditions in body. We measured the activity of natural killer cell and the data were shown in the Figure 3. The data indicated that celiac injection of

br-bFGF at the scheduled dose sharply increased activities of natural killer cells. Activities of natural killer cells from two experimental groups were markedly higher than that of control group.

Cells proliferation from spleen

Spleen is an important immune organ containing plenty of B and T lymphocytes whose proliferation is reflection of spleen immune function. Concanavalin A is an effective stimulator improving proliferation of T lymphocyte. This investigation employed concanavalin A to stimulate T cells proliferation and to resultantly reflect proliferation activity of spleen cells. To investigate the effects of bFGF on proliferation of spleen cells, cells were isolated and cultured in vitro after bFGF administration in vivo. The results showed that bFGF could significantly improve the proliferation of spleen cells in culture. As shown in Figure 4, the optical density values of the three bFGF treatment groups were higher than that of control group.

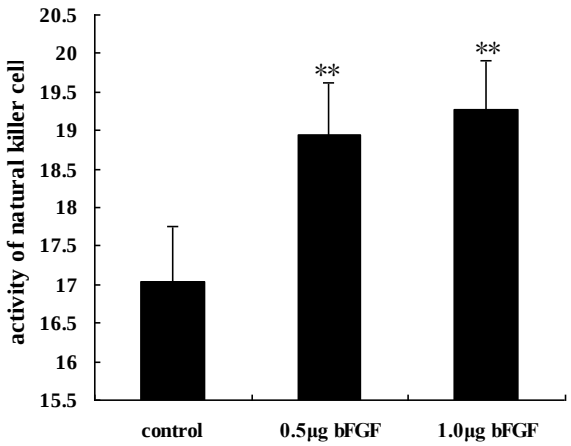

Figure 3: Effects of bFGF on activity of natural killer cells. Activities of natural killer cells in 0.5 μg and 1.0 μg bFGF groups were both significantly higher than that in normal saline control group, **P<0.01.

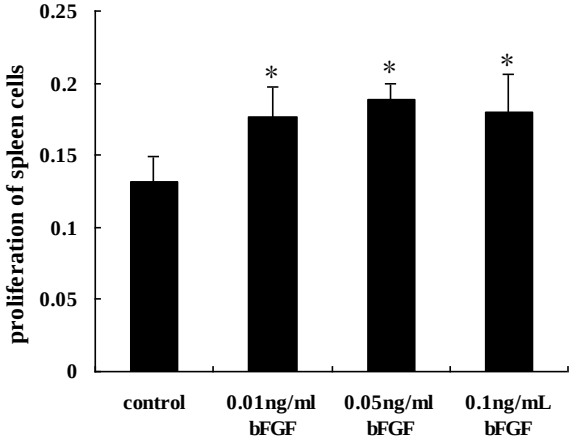

Figure 4: Effects of bFGF on proliferation of spleen cells.The data of three experimental groups were all significantly higher than that of control group, *P<0.05.

Discussion

Basic FGF is a multifunctional growth factor and large amounts of reports have been published in demonstration of its functions. A number of evidences have proved that macrophages and T lymphocyte can produce bFGF [6-8] but little is known about effects of bFGF on immunity in general. Our efforts in this study was to investigate the relationship of bFGF and immune responses and the major findings were the demonstration that following the administration of exogenous bFGF major immune activities in the body of experimental animal, such as number of natural killer cells, phagocytosis percentage and phagocytosis index of macrophage, number of antibody-produced cells and proliferation of spleen cells, were elevated significantly.

It has been proved that administration of bFGF can increase tissue factor expression in circulating macrophage, [14] and that immune neutralization of bFGF reduces the activity and number of macrophages.[5, 6] For further investigation of the effects of bFGF on macrophages in immune responses, we concentrated to evaluate macrophage inchondriosis that reflects immune activity of macrophages. Our results showed that administration of exogenous bFGF could significantly elevate phagocytosis percentage and phagocytosis index of celiac macrophages, which suggested that bFGF might perform an important role in innate immune.

Spleen is the major site of immune responses to blood-borne antigens and contains pools of several types of immune cells. Therefore, proliferation and transformation of spleen cells and plaque forming cells test of antibody-producing cells originated from B cells can reflect immunological function of T and B lymphocytes.[15] Previous studies have proved a connection between T lymphocytes or B cells and bFGF. [2, 9] However, there has been no evidence to indicate the effects of bFGF on B lymphocytes. To investigate whether both T cells and B cells are affected by bFGF, the proliferation of spleen cells and plaque forming cell were tested. Our results indicated that following the addition of bFGF spleen cells

proliferation was speeded significantly and the increasing of antibody-producing cells is more obvious than that of T lymphocytes. This fact may imply that effect of bFGF on humoral immune responses is more obvious than on cellular immune responses. In addition, along with the increase of concentration of exogenous bFGF, a decreasing tendency in the proliferation of spleen lymphocytes came forth. According to literature, during proliferation, differentiation, and/or malignant transformation, bFGF receptor expression level changes and this means that high concentration of bFGF may restrain the expression of bFGF receptor.[16-19] Suppression of expression of bFGF receptor would fade out the effect of bFGF on the proliferation of spleen lymphocytes.

To date, there is no evidence about effects of bFGF on natural killers. Our results revealed that natural killer cells were significantly affected by bFGF and their activities were boosted greatly under stimulation of bFGF.

Conclusion

In conclusion, as a multifunctional growth factor, bFGF not only exerts effects on mesoderm induction, angiogenesis, vessel wall, lung, hematopoiesis, nervous system, reproductive system, skin, eye, muscle, skeleton and digestive system, but also play its roles in regulation on the functions of immune system. Our findings are only tentative and much more work in this direction will be needed.

Conflict of Interest

Authors have no conflict of interest.

References

1. Dono R. Fibroblast growth factors as regulators of central nervous system development and function. Am J Physiol Integr Comp Physiol 2003; 284:867-881.

2. Bikfalvi A, Klein S, Pintucci G, Rifkin DB. Biological roles of fibroblast growth factor-2. Endo Rev 1997; 18:26-45.

3. Rifkin BD, Moscatelli D. Recent developments in the cell biology of basic fibroblast growth factor. J Cell Biol 1989;109:1-6.

4. Bouche G, Gas N, Prats H, Baldin V, Tauber JP, Teissie J, Amalric F. Basic fibroblast growth factor enters the nucleolus and stimulates the transcription of ribosomal genes in ABAE cells undergoing G0→G1 transition. Proc Natl Acad SciUSA 1987; 84:6770-6774.

5. Lefaucheur JP, Gjata B, Lafont H, Sebille A. Angiogenic and inflammatory responses follwing skeletal muscle injury are altered by immune neutralization of endogenous basic fibroblast growth factor,insulin-like growth factor-1 and transforming growth factor-β1. J Neuroimmunol 1996; 70:37-44.

6. Kobayashi S, Oktonori M, Kimura I, Kimura M. Interferon-γ-activated macrophages release interleukin-1α to increase tube formation from endothelial cells of rat aorta. Immunopharmacol 1995; 31:93-101.

7. Henke C, Marineili W, Jessurun J, Fox J, Harms D, Peterson M, Chiand L, Doran P. Macrophage production of basic fibroblast growth factor in the fibroproliferation disorder of alveolar fibrosis after lung injury. Am J Pathology 1993; 143:1189-1199.

8. Pugh-Humphreys RG. Macrophage-neoplastic cell interactions:implications for neoplastic cell growth. FEMS Microbiol Immunol 1992; 5:289-308.

9. Shen B, Arese M, Gualandris A, Rifkin DB. Intracellular association of FGF-2 with the ribosomal protein L6/TAREB107. Biochem Biophys Res Commun 1998; 252:524-528.

10. Liu HZ, Yang XY, Li Q, An XR, Chen YF, Zhong JP. Expression of bovine basic fibroblast growth factor gene in Escherichia coli and bioactivity of the expressed product. Vet Sci China. 2007; 37(10): 896-900.

11. Chen Q. Pharmacological research methods of Chinese medicine(secondly edition).People Health Publisher, Beijing 1996; pp84-85.

12. Ba DN. The Modern Immunological Technology and Application. Cooperative Publisher of Beijing Medical University & Xiehe Medical University of China, Beijing 1998; pp135-136.

13. Hu T, Geng PL. Effects of traditional Chinese medicine on the chemotherapeutical mouse cytokines and activity of natural killer cells. J Immunol China 2003; 19:616-617.

14. Corseaux D, Meurice T, Six I, Rugeri L, Ezekowitz MD, Rouvier P, Bordet R, Bauters C, Jude B. Basic fibroblast growth factor increases tissue factor expression in circulating monocytes and in vascular wall. Circulation 2000; 101:2000-2006.

15. Abbas AK, Lichtman AH. Cellular and molecular immunology(fifth edition). Library of Congress Cataloging-in-Publication Data 2003; pp1-40.

16. Moscatelli D. Autocrine downregulation of fibroblast growth factor receptors in F9 teratocarcinoma cells. J Cell Physiol 1994; 160:555-562.

17. Pertovaara L, Tienari J, Vainikka S, Partanen J, Sakseka O, Lehtonen E, Alitalo K. Modulation of fibroblast growth factor

receptor and signaling during retinoic acid-induced differebtiation of Tera-2 teratocarcinoma cells. Biochem Biophys Res Commun 1993; 191:149-156.

18. Mummery CL, van Rooyen M, Bracke M, van den Eijnden-van Raaij J, van Zoelen EJ, Alitalo K. Fibroblast growth factor-mediated growth regulation and receptor expression in embryonal carcinoma and embryonic stem cells and human germ cells. Biochem Biophys Res Commun 1993; 191:188-195.

19. Olwin BB, Hauschka SD. Cell surface fibroblast growth factor and epidermal growth factor receptor are permanently lost during skeletal muscle terminal differentiation in culture. J Cell Biol 1988; 107:761-769.