BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **224,** 33–36 (1996)
ARTICLE NO. 0980

Roles of Basigin, a Member of the Immunoglobulin Superfamily, in Behavior as to an Irritating Odor, Lymphocyte Response, and Blood–Brain Barrier

Tadahiko Igakura,*,† Kenji Kadomatsu,* Osamu Taguchi,‡ Hisako Muramatsu,* Tadashi Kaname,*,§ Teruo Miyauchi,‖ Ken-ichi Yamamura,§ Kimiyoshi Arimura,† and Takashi Muramatsu*,[1]

**Department of Biochemistry, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466, Japan; †The 3rd Department of Medicine, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890, Japan; ‡Laboratory of Experimental Pathology, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusa-ku, Nagoya 464, Japan; §Department of Developmental Genetics, Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine, 4-24-1 Kuhonji, Kumamoto 862, Japan; and ‖Japan Immunoresearch Laboratories, 351-1 Nishiyokote-cho, Takasaki, Gunma 370, Japan*

Received May 27, 1996

Basigin is a transmembrane glycoprotein belonging to the immunoglobulin superfamily. Using the knockout mouse lacking the basigin gene (*Bsg*), we analyzed the function of basigin in adult mice lacking the gene [*Bsg* (-/-)]. Although histochemical studies on the localization of basigin (also called HT7 and neurothelin) strongly indicated that it is involved in the function of the blood–brain barrier, basigin knockout mice showed only a little difference, if any, to wild-type mice in the function of the blood brain–barrier. The mitogenic response of lymphocytes upon mixed lymphocyte reaction was greater in *Bsg* (-/-) mice. Finally, *Bsg* (-/-) mice repeatedly visited filter paper impregnated with acetic acid or isozine, indicating an abnormality in either reception of the odor or behavior as to it.

The immunoglobulin superfamily comprises diverse proteins involved in intercellular recognition (1). Cell adhesion proteins (*i.e.* NCAM, myelin-associated glycoprotein and L1), growth factor receptors (*i.e.* FGF receptors) and molecules involved in antigen recognition (*i.e.* T cell receptors and major histocompatibility complex class I/II molecules) are typical immunoglobulin superfamily members.

Basigin is a unique member of the superfamily. Its protein portion is 27 kDa and its glycosylated form is 43 - 66 kDa (2). It is a transmembrane glycoprotein with two immunoglobulin domains. Basigin has been independently found in several laboratories and called by different names, such as gp 42 (3), HT-7(4), neurothelin (5, 6), leukocyte activation antigen M6 (7), and EMMPRIN (8). Importantly, basigin has been repeatedly implicated in the function of the blood brain barrier, since in the brain, it is preferentially located in capillaries (4-6). Furthermore, basigin becomes newly expressed upon lymphocyte activation (7).

In order to understand the *in vivo* function of basigin, we produced knockout mice lacking the basigin gene (*Bsg*) (9). The null mutant embryos mainly died around the time of implantation (9). A small number of surviving adult mice were sterile, indicating that the basigin gene is important in development and reproduction (9). In this study, we investigated the function of basigin in adult mice using the surviving knockout mice.

[1] Corresponding author. Fax: 81-52-744-2065.

Abbreviations: ES, embryonic stem; mAbs, monoclonal antibodies; NCAM, neural cell adherion molecule; TCR, T cell receptor.

0006-291X/96 $18.00

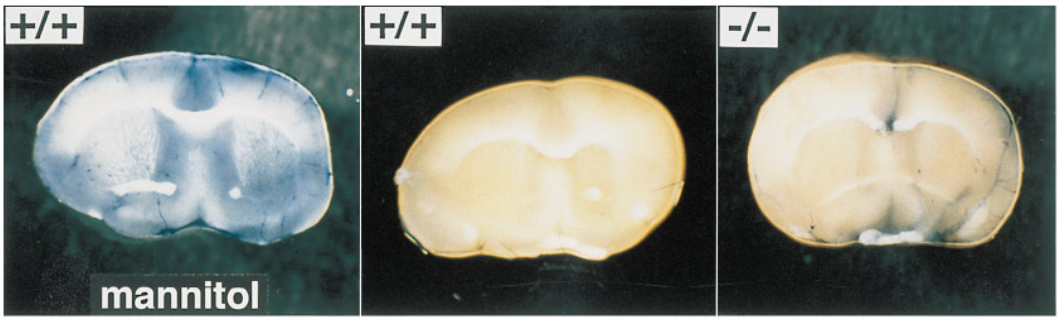

FIG. 1. Examination of the integrity of the blood–train barrier by the Evans blue dye method. Each brain was removed after perfusion with Evans blue dye and examined. (+/+) Mannitol ; a brain from the wild-type littermates treated with mannitol. (+/+) A brain from the mid-type littermates. (−/−) A brain of the knockout mice. Cornal sections of the whole brain are shown.

MATERIALS AND METHODS

The basigin gene (Bsg) knockout mice. In the knockout mice, 0.2kb of exon 1 of the *Bsg* was replaced with the neomycin resistance gene by homologous recombination (9). The ES cells used were D3 and blastocysts used for injection were from C_{57}BL/6. Male chimeric mice were mated with C_{57}BL/6. F1 mice with the recombined genome were mated with each other to yield null mutant mice (9). Southern blot analysis confirmed that *Bsg* was deleted in both chromosomes in the null mutant mice and that no random integration or gross rearrangement occurred in the mice. Northern and Western blot analyses also comfirmed that basigin mRNA and the protein were absent in the mutant.

Evans blue dye testing of the integrity of the blood–brain barrier. The blood–brain barrier activity was evaluated by means of Evans blue dye method as described by Inoue et al. (10), and Thumwood et al. (11). Briefly, 200 μl of a 2% solution of Evans blue in saline was intravenously injected into mice via the tail. After 1 h, the mice were anesthetized with Nembutal and their brains were perfused with 10 ml of normal saline through the left cardial ventricle. Then each brain was examined as to whether the blue dye had infiltrated into the grey matter due to destruction of the blood brain barrier.

Serological analysis. Analysis of cell surface antigens was performed with a FACScan (Becton Dickinson, Mountain View, CA) using monoclonal antibodies (mAbs) to B220, CD3, CD4, CD8, TCR α β , and TCR V β 6, 8 and 11 (Pharmingen, San Diego, CA). mAbs were directly conjugated with FITC or biotin. Phycoerythrin-streptavidin (Biomeda, Foster City, CA) was used as the secondary reagent.

Mixed lymphocyte culture assay. Spleen cells were taken from 3 different *Bsg(−/−)* mice or littermates. The cells (5×10^5) were cocultured with irradiated (24 Gy, Hitachi MBR-1520R; Hitachi) spleen cells (5×10^5) of BALB/c, C3H/He or C_{57}BL/6 mice in 0.2 ml of RPMI 1640 with 10 % fetal calf serum for 4 days at 37 °C, followed by 16 h incubation in the presence of 18.5 kBq [^{3}H] thymidine. Cells were harvested on a filter with LABO Mash (Lab Science Inc., Tokyo, Japan) and precipitated with trichloroacetic acid. The amount of [^{3}H]thymidine in the precipitate was determined.

Behavior toward an irritating odor. A filter (1×1cm) was impregnated with 0.5 ml of 100 % acetic acid or a 10 % isodine solution and placed in the center of the floor of a 20 × 30 cm cage. A 4 cm circle, whose center coincided with the filter paper, was marked on the floor. A mouse was placed in the cage and was observed for 10 min to determine the number of ''visits'' to the filter paper. When the nose of the mouse came within the 4 cm circle, it was counted as one visit.

RESULTS AND DISCUSSION

Blood–brain barrier in the knockout mice. We employed the Evans blue dye test to examine the integrity of the blood–brain barrier. When the blood–brain barrier is destroyed, the dye stains the brain extensively. As a positive control, we used mannitol-perfused mice, whose blood–brain barrier was destroyed. The brains of the latter were strongly stained, while the brains of normal mice were not stained (Fig. 1). The brains of the basigin gene knockout mice [*Bsg* (−/−) mice] showed no staining in two cases and a little staining in one case (Fig. 1; −/−). Thus, we could not detect any significant defect in the blood brain–barrier of the mutant

TABLE 1
Proliferative Response to Allogeneic Spleen Cells in a Mixed Lymphocyte Culture[a]

Responder	Stimulator			
	BALB/c H-2^d	C3H/He H-2^k	C_{57}BL/6 H-2^b	None
Wild [*Bsg* (+/+)]				
(H-2^b)	8,000	19,000	900	800
	9,000	9,400	1,100	1,100
	10,700	9,700	2,100	2,300
Knockout [*Bsg* (−/−)]				
(H-2^b)	28,100	32,300	3,700	3,000
	20,600	17,800	2,800	2,500
	24,200	23,900	3,200	2,000

Note. For each responder/stimulator pair, 3-assays were performed using 3 different wild or knockout mice.
[a] The extent of thymidine incorporation was measured.

mice, even though localization of the molecule strongly indicated its involvement in the function. Basigin exhibits over-all homology with embigin, another member of the Ig superfamily (12, 13). Since the localization of embigin in the adult brain is not known, there remains a possibility that embigin compensated for the loss of basigin in the mutant mice.

Altered lymphocyte mitogenicity in the mutant mice. Basigin is considered to be a leukocyte activation antigen, since it is not expressed in normal lymphocytes, but is expressed in Con A-stimulated lymphocytes (6). Leukocytes of rheumatoid arthritis patients show more neutrophils exhibiting basigin expression than ones of normal human subjects (6).

The surface phenotype of spleen cells in the knockout and wild-type mice were analyzed with a FACS using the following mAbs: B220, CD3, CD4, CD8, TCR α β, and TCR V β 6, 8 and 11. There were, however, no apparent differences between these two groups of mice (data not shown). Then, we examined possible difference in the lymphocyte response. We found that mixed lymphocyte reaction of lymphocytes from *Bsg* (−/−) mice were significantly greater than that of lymphocytes from wild-type littermates (Table 1). The mixed lymphocyte reaction is a T cell reaction toward lymphocytes with different H-2 haplotypes. Therefore, it is likely that basigin is a molecule which newly appears on the surface of activated lymphocytes and suppresses the activation of neighbouring lymphocytes through intercellular recognition.

Abnormal response to an irritating odor. We placed a filter impregnated with acetic acid in the center of a cage and then observed the behavior of mice as to the paper. The knockout mice repeatedly visited the paper and sniffed it, while wild type littermate tended to stay away from the paper (Fig. 2). A similar tendency was found for the behavior as to a filter paper impregnated with isogine, a typical compound with an irritating odor (Fig. 2). Therefore, the knockout mice are abnormal in the behavior toward an irritating odor. Since we found expression of basigin mRNA in brain neurons of the mouse (Fang et al., manuscript in preparation), one possible explanation is an abnormality in the perception of the odor signal at the brain level. These results clearly indicate that basigin is not only an important molecule regulating development and reproduction (9), but also a molecule necessary for normal adult life, since it controls at least the response to an irritating odor and lymphocyte response to external signals. The pleiotrophic function of this cell surface molecule is consistent with the view that the immunoglbulin domain of basigin is structurally related to putative primordial form of the immunoglibulin domain before the evolution of the C and V domains (2).

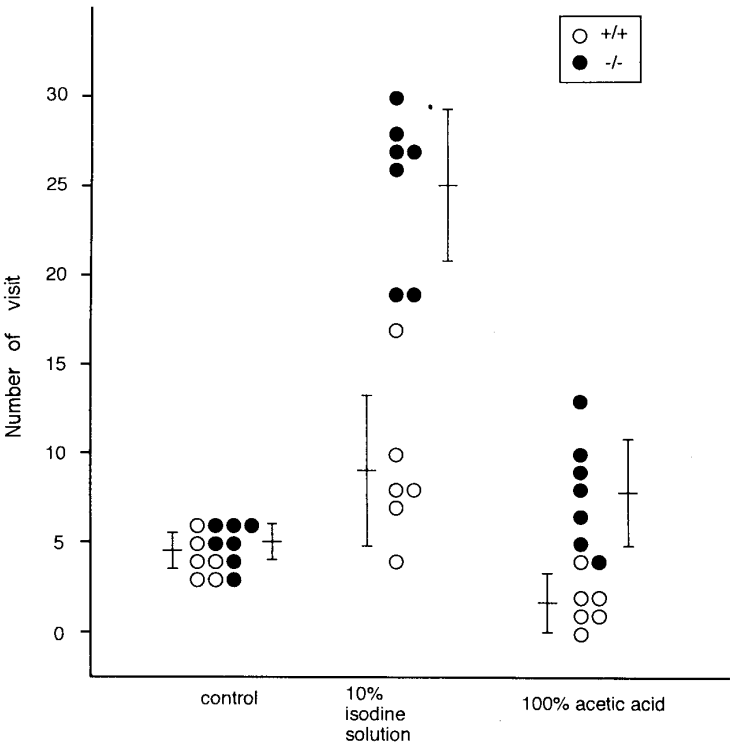

FIG. 2. Difference in behavior as to an irritating odor between *Bsg* (−/−) mice and wild-type littermates. Seven *Bsg* (−/−) mice and six wild-type littermates were used. Bars indicate standard deviation. Control: the filter paper was impregnated with water.

ACKNOWLEDGMENTS

We thank Ms. Kinuko Takamiya and Keiko Yoshioka for secretarial assistance. This work was supported by grants from the Ministry of Education, Science and Culture of Japan and Science and Technology Agency, Japan.

REFERENCES

1. Williams, A. F., and Barclay, A. N. (1988) *Ann. Rev. Immunol.* **6,** 381–405.
2. Miyauchi, T., Kanekura, T., Yamaoka, A., Ozawa, M., Miyazawa, S., and Muramatsu, T. (1990) *J. Biochem.* **107,** 316–323.
3. Altruda, F., Cervella, P., Gaeta, M. L., Daniele, A., Giancotti, F., Tarone, G., Stefanuto, G., and Silengo, L. (1989) *Gene* **85,** 445–452.
4. Seulberger, H., Lottspeich, F., and Risau, W. (1990) *EMBO J.* **9,** 2151–2158.
5. Schlosshauer, B., and Herzog, K.-H. (1990) *J. Cell Biol.* **110,** 1261–1274.
6. Seulberger, H., Unger, C. M., and Risau, W. (1992) *Neurosci. Lett.* **140,** 93–97.
7. Kasinrerk, W., Fiebiger, E., Stefanová, I., Baumruker, T., Knapp, W., and Stockinger, H. (1992) *J. Immunol.* **149,** 847–854.
8. Biswas, C., Zhang, Y., DeCastro, R., Guo, H., Nakamura, T., Kataoka, H., and Nabeshima, K. (1995) *Cancer Res.* **55,** 434–439.
9. Igakura, T., Kadomatsu, K., Kaname, T., Muramatsu, H., Miyauchi, T., Fan, Q.-W., Takahashi, M., Taguchi, O., Senda, T., Kuno, N., Yamamura, K., Arimura, K., and Muramatsu, T. (1996) submitted.
10. Inoue, T., Fukui, M., Nishio, S., Kitamura, K., and Nagara, H. (1987) *J. Neurosurg.* **66,** 256–263.
11. Thumwood, C. M., Hunt, N. H., Clark, I. A., and Cowden, W. B. (1988) *Parasitology* **96,** 579–589.
12. Ozawa, M., Huang, R. P., Furukawa, T., and Muramatsu, T. (1988) *J. Biol. Chem.* **263,** 3059–3062.
13. Huang, R. P., Ozawa, M., Kadomatsu, K., and Muramatsu, T. (1993) *Dev. Biol.* **155,** 307–314.