THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 26, pp. 21663–21672, June 22, 2012

Independent Recognition of *Staphylococcus aureus* by Two Receptors for Phagocytosis in *Drosophila*[*] [S]

Received for publication, December 14, 2011, and in revised form, April 20, 2012 Published, JBC Papers in Press, April 30, 2012, DOI 10.1074/jbc.M111.333807

Akiko Shiratsuchi[‡§¶1], Toshinobu Mori[§], Kenji Sakurai[¶], Kaz Nagaosa[‡§¶], Kazuhisa Sekimizu[‖], Bok Luel Lee[**], and Yoshinobu Nakanishi[‡§¶2]

*From the [‡]Graduate School of Medical Sciences, [§]School of Pharmacy, and [¶]Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan, the [‖]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan, and the **College of Pharmacy, Pusan National University, Geumjeong-Gu, Busan 609-735, Korea*

Background: How multiple receptors for phagocytosis contribute to host immunity against bacterial infection has been elusive.
Results: *Drosophila* phagocytes use two receptors, integrin and Draper, to effectively recognize and engulf *Staphylococcus aureus*.
Conclusion: Dual recognition system exists in *Drosophila* for phagocytic removal of pathogenic bacteria.
Significance: Study of cellular immune response is important for understanding host defense against infectious diseases.

Integrin βν, one of two β subunits of *Drosophila* integrin, acts as a receptor in the phagocytosis of apoptotic cells. We here examined the involvement of this receptor in defense against infection by *Staphylococcus aureus*. Flies lacking integrin βν died earlier than control flies upon a septic but not oral infection with this bacterium. A loss of integrin βν reduced the phagocytosis of *S. aureus* and increased bacterial growth in flies. In contrast, the level of mRNA of an antimicrobial peptide produced upon infection was unchanged in integrin βν-lacking flies. The simultaneous loss of integrin βν and Draper, another receptor involved in the phagocytosis of *S. aureus*, brought about a further decrease in the level of phagocytosis and accelerated death of flies compared with the loss of either receptor alone. A strain of *S. aureus* lacking lipoteichoic acid, a cell wall component serving as a ligand for Draper, was susceptible to integrin βν-mediated phagocytosis. In contrast, a *S. aureus* mutant strain that produces small amounts of peptidoglycan was less efficiently phagocytosed by larval hemocytes, and a loss of integrin βν in hemocytes reduced a difference in the susceptibility to phagocytosis between parental and mutant strains. Furthermore, a series of experiments revealed the binding of integrin βν to peptidoglycan of *S. aureus*. Taken together, these results suggested that Draper and integrin βν cooperate in the phagocytic elimination of *S. aureus* by recognizing distinct cell wall components, and that this dual recognition system is necessary for the host organism to survive infection.

Phagocytosis is a cellular immune response by which cells foreign to host organisms are incorporated and digested by immune cells called phagocytes (1, 2). In innate immunity, phagocytosis plays a front-line role in the host defense against invasive microorganisms. The importance of phagocytosis relative to humoral immune responses in innate immunity, such as the production of antimicrobial substances, has been controversial and in *Drosophila melanogaster* appears to depend on infectious state: the two immune responses sometimes cooperate with each other to prevent infectious diseases (3, 4) or act differentially depending on the stage of infection (5) and the kind of microorganism (6). Another important issue is whether the occurrence of phagocytosis is necessary for the subsequent induction of humoral responses, with reports both for (7–10) and against (11, 12) this notion. To resolve these issues, it is necessary to deepen our understanding of the mechanism of phagocytosis, and in particular, how invading microorganisms are recognized and engulfed by phagocytes.

It is widely appreciated that the fundamental mechanism of immune response is common among species from *Drosophila melanogaster* and *Caenorhabditis elegans*, animals frequently used as models in the study of innate immunity (13), to mice and humans (14–16). The use of *Drosophila* provides the advantage that genetically tractable experiments are feasible using whole animals infected by either injury or feeding with microorganisms (14). There are three types of *Drosophila* blood cells, *i.e.* hemocytes, which play distinct roles in innate immunity, namely, plasmatocytes, crystal cells, and lamellocytes, with plasmatocytes responsible for the phagocytic elimination of invading microorganisms (15–17). In mammals, the phagocytosis of bacteria is accomplished mostly with the aid of a serum component called opsonin that connects target cells and phagocytes. Both opsonin-dependent and -independent mechanisms of phagocytosis are likely to exist in *Drosophila*, but the former is less clearly understood (18). In contrast, a number of candidate receptors for the phagocytosis of bacteria have been reported, which are presumed to directly recognize bacterial

[*] This work was supported by Grants-in-Aid for Scientific Research from Japan Society for the Promotion of Science (Grant Numbers 20570127 and 23570160, to A. S.; 22370049, to Y. N.), Hayashi Memorial Grant for Female Scientists (to A. S.), a grant from Astellas Foundation for Research on Metabolic Disorders (to A. S.), a grant from Danone Foundation (to A. S.), and an institutional research grant from Kanazawa University (to A. S.).
[S] This article contains supplemental Fig. S1.
[1] To whom correspondence may be addressed: Shizenken, Kakuma-machi, Kanazawa, Ishikawa 920-1192, Japan. Tel.: 81-76-234-4481; Fax: 81-76-234-4480; E-mail: ashira@staff.kanazawa-u.ac.jp.
[2] To whom correspondence may be addressed: Shizenken, Kakuma-machi, Kanazawa, Ishikawa 920-1192, Japan. Tel.: 81-76-234-4481; Fax: 81-76-234-4480; E-mail: nakanaka@p.kanazawa-u.ac.jp.

components, including peptidoglycan recognition protein LC (19); members of the scavenger receptor family of proteins such as Peste (20), dSR-CI (21), and Croquemort (22); and the Nimrod family of proteins including Eater (6, 23, 24), Nimrod C1 (25) and Draper (26). However, the identity of putative cell wall components serving as ligands for these receptor proteins is largely obscure. Nevertheless, it is anticipated that a multiple recognition system for the phagocytic removal of bacteria exists in *Drosophila* to protect host organisms from infection with pathogenic microorganisms. Croquemort (27, 28) and Draper (29, 30) were originally found to act as receptors in the phagocytosis of apoptotic cells in *Drosophila*, suggesting that common receptors are used in the phagocytic elimination of apoptotic cells and invading bacteria in *Drosophila*. We recently reported that integrin $\beta\nu$, a β subunit of *Drosophila* integrins, is required for the phagocytic elimination of apoptotic cells in *Drosophila* embryos (31). In the present study, we examined whether this subunit is also involved in host defense against microorganisms.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Fly Stocks, and Antibody—The strain RN4220 was used as wild-type *S. aureus* and the parental strain for M0674 and TS2901. The strain M0674 does not express *ltaS* that codes for polyglycerolphosphate synthase, an enzyme responsible for the synthesis of polyglycerolphosphate of lipoteichoic acid, and thus lacks lipoteichoic acid (32). The strain TS2901 possesses a temperature-sensitive *murB* that codes for UDP-*N*-acetylenolpyruvylglucosamine reductase, an enzyme responsible for the synthesis of peptidoglycan, and produces a reduced level of peptidoglycan at a non-permissive temperature (33). Complementation of TS2901 with wild-type *murB* was done according to standard procedures using the plasmid pND50 as a vector (33). The strains W3110 and 168 were used as wild-type *Escherichia coli* and *Bacillus subtilis*, respectively. The bacteria were cultured at 30 °C (*S. aureus* strains M0674 and TS2901) or 37 °C (all other *S. aureus*, *E. coli*, and *B. subtilis* strains) with Luria-Bertani medium supplemented with glucose and antibiotics wherever required. When the cultures reached full growth, the bacteria were harvested, washed with PBS, and used in the experiments. The following lines of *Drosophila* were used in this study: w^{1118} (Bloomington Drosophila Stock Center, Indiana University, Bloomington, IN), *betaInt-nu*^{1} (34), *betaInt-nu*^{2} (34), $drpr^{\Delta 5}$ (29), $elmo^{KO}$ (35), and $Rac1^{J11}$ $Rac2^{\Delta}$ (4) (Bloomington Drosophila Stock Center). Some of the fly lines were used after changing balancers, and other lines were generated through the mating of existing flies. The anti-integrin $\beta\nu$ antiserum was raised by immunizing rats with an extracellular region (amino acid positions 6–697 with the amino terminus numbered 1) of integrin $\beta\nu$ that had been expressed in *E. coli* as a protein fused to GST at the amino terminus and purified to homogeneity. Antigen specificity of this antibody was confirmed in Western blotting (supplemental Fig. S1). Anti-GST mAb and HRP-conjugated anti-mouse IgG antibody were purchased from Millipore and GE Healthcare, respectively.

Bacterial Infection, and Assays for Bacterial Growth and Antimicrobial Peptide Production—The injection of bacteria into the abdomen of male adult flies, reminiscent of a septic infection, was done according to established procedures (37) with modifications (26). Briefly, flies 3–7 days after eclosion (15–20 flies per vial, 1–3 vials in each experiment) were injected with 50 nl of PBS containing given numbers of bacteria (1×10^3 per fly except for the analysis of phagocytosis, see below). For the oral infection, adult flies (13–15 flies per vial, 3 vials in each experiment) that had been dehydrated for 2 h without food were maintained in a vial containing filter paper soaked with 0.3 ml of 1% (w/v) sucrose including about 10^{10} bacteria or 50 mM H_2O_2 according to a published procedure (38). The flies infected with bacteria were maintained at 29 °C until use. The growth of bacteria in flies was analyzed by determining the colony-forming activity of injected bacteria as described previously (26). Briefly, homogenates of infected flies were plated at serial dilutions on an agar medium, and the number of colonies that appeared after incubation was expressed as cfu per fly. The level of mRNA of drosomycin was determined by quantitative RT-PCR as described previously (26). In brief, total RNA was prepared from about 100 infected flies and used as a template in RT to synthesize cDNA. The cDNA was then used as a template in real-time PCR for amplifying the sequences corresponding to mRNA of drosomycin and ribosomal protein 49, and the levels of the signals derived from drosomycin mRNA were normalized against that from ribosomal protein 49 mRNA, which served as an internal control. The DNA oligomers used as primers in real-time PCR were: 5′-CGTGAGAACCTTTTCCAATATGATG-3′ (forward) and 5′-TCCCAGGACCACCAGCAT-3′ (reverse) for drosomycin; and 5′-GACGCTTCAAGGGACAGTATCTG-3′ (forward) and 5′-AAACGCGGTTCTGCATGAG-3′ (reverse) for ribosomal protein 49.

Assay for Phagocytosis—An assay for the phagocytosis of *S. aureus in vivo* and *in vitro* was carried out as described previously (26). Briefly, for the assay *in vivo* male adults were injected with *S. aureus* (1×10^6 per fly), which had been labeled with FITC or 5-carboxyfluorescein, succinimidyl ester (Molecular Probes), maintained at 29 °C for 2 h, administered with PBS containing 0.4% (w/v) trypan blue to quench fluorescence derived from bacteria left unengulfed, and examined under a fluorescence microscope after 30 min. The level of phagocytosis was determined based on the size of clusters of fluorescent materials, which corresponded to aggregates of hemocytes that had engulfed bacteria. For the assay *in vitro*, larval hemocytes were prepared essentially according to established procedures (37) with modifications (26). In brief, hemolymph was collected from wandering third-instar larvae, placed on Teflon-coated glass slides, and incubated on ice for 10 min so that isolated hemocytes adhered to the slides. The hemocyte cultures were incubated with FITC-labeled bacteria (hemocytes:targets = 1 : 500) or fluorescence-labeled latex beads (2.0 μm in diameter; Molecular Probes) (hemocytes:targets = 1 : 200) at 25 °C for 10–30 min, washed with PBS, enclosed with 0.4 mM sodium acetate buffer (pH3.5) containing 0.4% trypan blue, and examined by fluorescence microscopy. The ratio of hemocytes containing target particles and the number of target particles contained in 100 phagocytes were determined

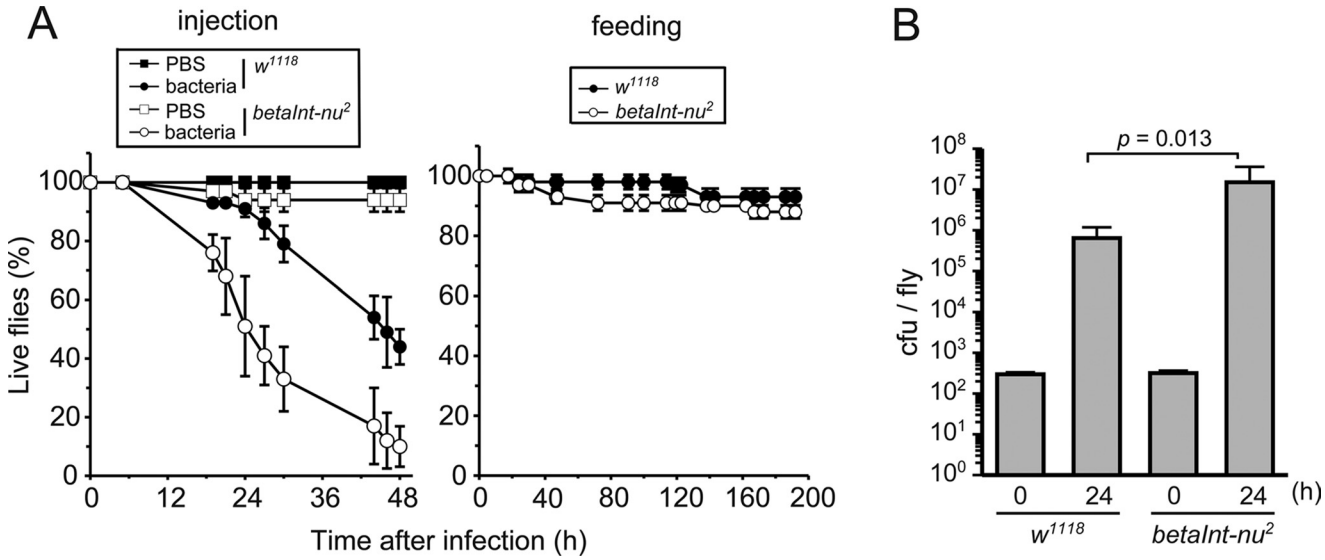


FIGURE 1. **Requirement of integrin βν for host defense against septic infection with *S. aureus*.** Adult flies of control (w^{1118}) (*closed symbols*) and integrin βν-deficient (*betaInt-nu*^{2}) (*open symbols*) lines were infected with wild-type *S. aureus* (strain RN4220) by injury (injection at the abdomen) (*left* in *A* and *B*) or feeding (*right* in *A*), and subjected to an analysis of fly survival (*A*) and bacterial growth (*B*) at the indicated periods. The square symbols in the *left panel* of *A* denote the results for flies injected with solvent (PBS) alone. In *A*, data from one of three independent experiments that yielded similar results are presented. In *B*, cfu values at 24 h relative to those at 0 h were compared between w^{1118} and *betaInt-nu*^{2}. Genotype of the fly line analyzed is *w; betaInt-nu*^{2} (*betaInt-nu*^{2}).

and exhibited as "phagocytosing hemocytes" and "engulfed targets," respectively.

Assays for Binding of Peptidoglycan to Hemocytes, and Integrin to Bacteria and Peptidoglycan—To examine the binding of peptidoglycan to hemocytes, larval hemocytes (1×10^4) were incubated for 10 min at 25 °C with an insoluble preparation of peptidoglycan (20 μg), which had been prepared from wild-type *S. aureus* (39) and labeled with FITC. The cells were then washed, fixed, and examined by fluorescence microscopy. For the analysis of the binding of integrin βν to *S. aureus*, a mixture of GST-fused integrin βν (10 pmol) used as an antigen for generating the antibody and GST alone (10 pmoles) was incubated with strains of *S. aureus* (1×10^8) for 15 min at room temperature, and the bacteria were collected by centrifugation, washed with PBS, lysed, and analyzed by Western blotting with anti-GST mAb, essentially as described previously (31). Binding of integrin βν to peptidoglycan was tested using insoluble and soluble fractions of peptidoglycan. A mixture of GST-fused integrin βν (10 pmol) and GST (10 pmol) was incubated with insoluble fractions of peptidoglycan prepared from *E. coli* (5 μg) (40) or *S. aureus* (wild-type strain) (40 μg) for 5 min at room temperature, and peptidoglycan was precipitated by centrifugation, washed with PBS, and analyzed by Western blotting using anti-GST mAb. To examine the binding of integrin βν to soluble peptidoglycan preparations, dishes of a 96-well culture container (MS-8496F; Sumitomo Bakelite, Tokyo, Japan) were coated with peptidoglycan (4 μg per well), which had been prepared from wild-type *S. aureus* (39), and increasing amounts of GST-fused integrin βν or GST were added in triplicate. The dishes were incubated for 3 h at room temperature, washed, successively supplied with anti-GST mAb and HRP-conjugated anti-mouse IgG antibody, and subjected to a colorimetric reaction using *o*-phenylenediamine as a substrate, and the amount of the reaction products was determined by measuring A_{490}.

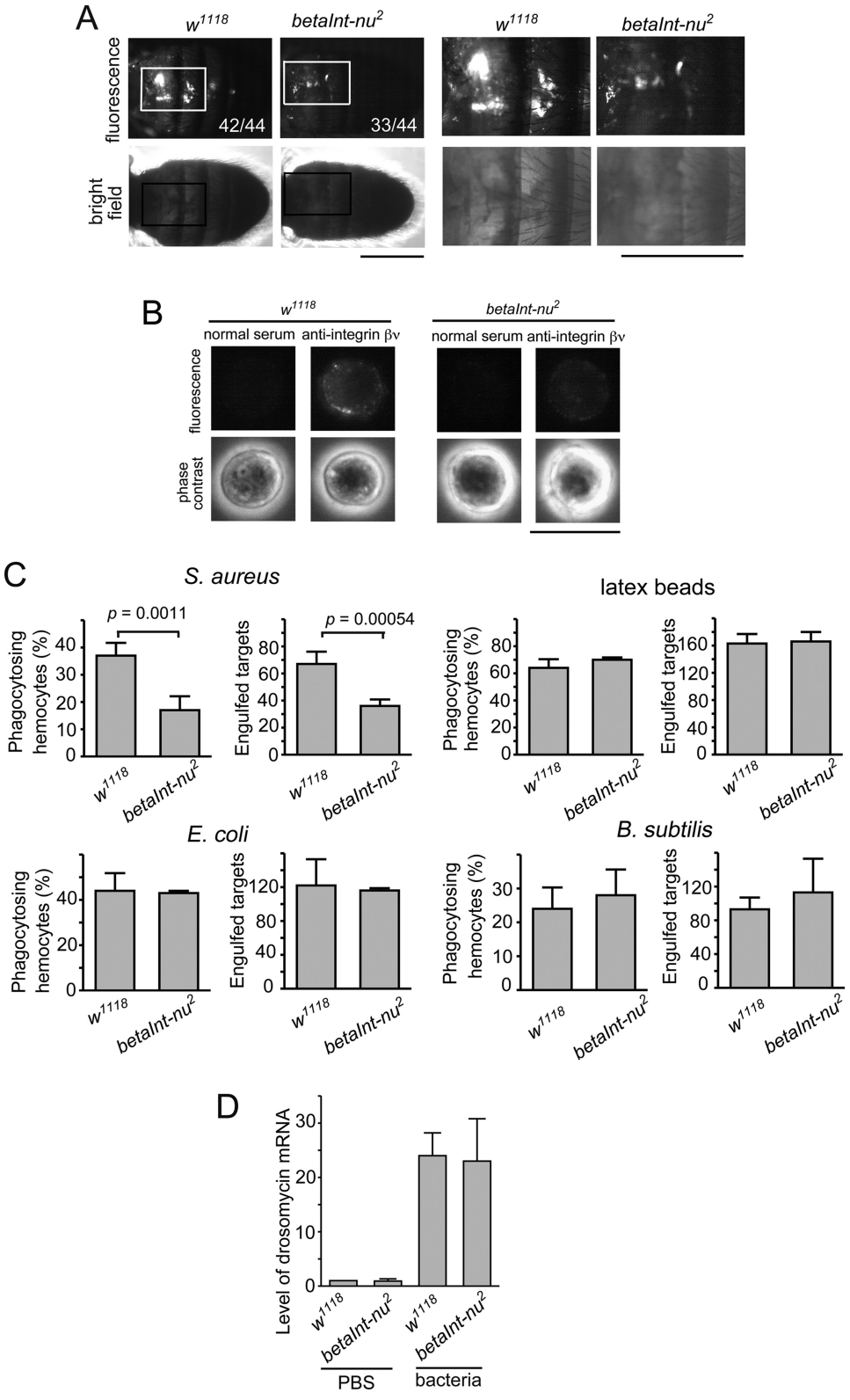
Data Processing and Statistical Analysis—Results from quantitative analyses are expressed as the mean ± S.D. of the data from at least three independent experiments, unless otherwise stated in the text. Other data are representative of at least three independent experiments that yielded similar results. Statistical analyses were performed using Student's *t* test, and *p* values of less than 0.05 were considered significant and are indicated in the figures.

RESULTS

Role for Integrin βν in Phagocytosis of S. aureus by Drosophila hemocytes—We first examined the role of integrin βν in the defense of *Drosophila* against *S. aureus*. For this purpose, adult flies of an integrin βν-lacking mutant (*betaInt-nu*^{2}) were subjected to an abdominal or oral infection with *S. aureus*, and the ratio of live flies was determined (Fig. 1*A*). As we showed previously (26), the abdominal injection of the bacterium led to the death of control flies (w^{1118}) while feeding flies with bacteria-containing meals did not have a lethal effect. Under these conditions, a loss of integrin βν accelerated the death of flies that had received the abdominal infection but did not influence the consequence of the oral infection with or without superoxide (data not shown), which enhances the killing of flies orally infected with *S. aureus* (38). There was no difference in survival between control flies and the integrin βν-lacking mutant that had received an injection with PBS, the solvent for bacteria. These results indicated the involvement of integrin βν in the mechanism to combat invading *S. aureus*. We then examined if a loss of integrin βν helps the growth of *S. aureus* in adult flies. The number of colony-forming bacteria recovered from flies 24 h after the infection was determined and compared between the mutant and control flies. More colony-forming bacteria were obtained from integrin βν-lacking flies (Fig. 1*B*), suggest-

ing the inhibitory effect of integrin $\beta\nu$ on bacterial growth in adult flies.

We next examined which type of immune response, cellular or humoral, integrin $\beta\nu$ participates in. An assay for phagocytosis, a cellular response, was first conducted *in vivo* using fluorescence-labeled *S. aureus* injected into the abdomen of adult flies. We found that the size of fluorescent clusters, a hallmark of the engulfment of bacteria by hemocytes, was smaller in integrin $\beta\nu$-lacking flies than in control flies (Fig. 2*A*). To further confirm this, we determined the phagocytic activity of hemocytes isolated from third-instar larvae *in vitro*. When larval hemocytes were first immunocytochemically analyzed for



the presence of integrin βν, most (>95%) cells were positive for punctate signals around surfaces, which were almost lost in the null mutant (Fig. 2*B*). The level of phagocytosis of *S. aureus* with integrin βν-lacking hemocytes was about half of that with control hemocytes while latex beads were phagocytosed equally by either hemocyte preparation (Fig. 2*C*). A decreased level of phagocytosis upon the loss of integrin βν was replicated in experiments both *in vivo* and *in vitro* with another null allele of *betaInt-nu* (*betaInt-nu*^{1}) (data not shown). In contrast, *E. coli* and *B. subtilis* were effectively phagocytosed by larval hemocytes regardless of the absence of integrin βν (Fig. 2*C*), suggesting that hemocytes do not use this integrin subunit for phagocytosing these bacteria. The level of mRNA of drosomycin, an antimicrobial peptide produced as an innate immune response to bacterial infection via the Toll pathway (15), was not altered by the loss of integrin βν (Fig. 2*D*). These results indicated that integrin βν is required for the phagocytic elimination of invading *S. aureus*, most likely as a receptor for phagocytosis, but not for the production of antimicrobial peptides.

Independent Actions of Integrin βν and Draper in Defense against S. aureus—We previously reported that Draper acts as a receptor in the phagocytosis of *S. aureus* in *Drosophila* (26). The functional relationship of integrin βν with Draper was determined because integrin βν also likely acts as a receptor for phagocytosis. We determined the level of phagocytosis with flies lacking both integrin βν and Draper, and compared it to that with flies deficient in either protein alone. In an assay *in vivo*, the level of phagocytosis in double-mutant flies seemed to be somewhat lower than that in single-mutants (Fig. 3*A*). This was more significant when larval hemocytes prepared from mutant flies were examined for their phagocytic activity *in vitro*. The level of phagocytosis by hemocytes lacking either integrin βν or Draper was 60–70% of that by control hemocytes while it was reduced to about 30% of the control level upon a simultaneous loss of the two receptors (Fig. 3*B*). These results indicated that both integrin βν and Draper are required for a maximal level of the phagocytosis of *S. aureus* by *Drosophila* hemocytes. The *S. aureus* gene *ltaS* codes for an enzyme responsible for the synthesis of glycerolphosphate repeat of lipoteichoic acid, and bacteria that do not express *ltaS* lack lipoteichoic acid (32). Draper requires lipoteichoic acid for the efficient phagocytosis of *S. aureus*: hemocytes of control flies phagocytose *ltaS*-deficient *S. aureus* less effectively than parental bacteria while Draper-lacking hemocytes almost equally phagocytose these two bacterial strains at a lower level (26). We found that hemocytes lacking integrin βν required the presence of lipoteichoic acid in *S. aureus* for a maximal level of phagocytosis (Fig. 3*C*), suggesting that the integrin βν-mediated phagocytosis of *S. aureus* does not depend on lipoteichoic acid. The fact that Draper recognizes lipoteichoic acid as a ligand for phagocytosis (26) suggested that integrin βν recognizes a cell wall component(s) of *S. aureus* other than lipoteichoic acid. We next examined the cooperation between the two proteins in defense mechanisms besides phagocytosis. The level of bacterial growth was higher in the double-mutant than single-mutants (*left panel* in Fig. 3*D*), and flies lacking both integrin βν and Draper died earlier than those deficient in the expression of either protein (*right panel* in Fig. 3*D*). The above-described data collectively showed that integrin βν and Draper cooperate with each other for flies to survive infection with *S. aureus*.

Role for Peptidoglycan in Integrin βν-mediated Phagocytosis of S. aureus—It is likely that a cell wall component other than lipoteichoic acid acts as a ligand for integrin βν. In a previous study, we determined the level of phagocytosis of various *S. aureus* strains with defects in the synthesis of cell walls, and found teichoic acid and glycolipids but not lipoproteins to be required for efficient phagocytosis (26). Lipoteichoic acid was later found to serve as a ligand for Draper (26), and the involvement of peptidoglycan was yet to be examined. We therefore wondered if peptidoglycan is involved in the integrin βν-mediated phagocytosis of *S. aureus*. A strain of *S. aureus* possessing a temperature-sensitive *murB* (strain TS2901) produces a reduced level of peptidoglycan at a non-permissive temperature (33). This mutant strain behaved as a less effective target for phagocytosis than the parental strain in an assay *in vivo* (Fig. 4*A*) as well as *in vitro* (Fig. 4*B*). Complementation of the mutant strain with the wild-type gene almost completely restored this defect (Fig. 4, *A* and *B*). These results indicated that the expression of *murB* is necessary for *S. aureus* to be effectively phagocytosed by *Drosophila* hemocytes. We then examined the phagocytosis of the *murB*-mutant and parental strains by hemocytes prepared from integrin βν-lacking and control (w^{1118}) flies (Fig. 4*C*). The *murB*-mutant strain was still less effectively phagocytosed than the parental strain by hemocytes lacking integrin βν. However, a difference in the susceptibility to phagocytosis between the two *S. aureus* strains was significantly smaller in experiments using integrin βν-lacking hemocytes compared with that with control hemocytes. These results suggested the involvement of peptidoglycan in the integrin βν-mediated phagocytosis of *S. aureus*.

We next asked if integrin βν binds peptidoglycan. To test the binding of peptidoglycan to hemocytes, a fraction of the cell wall rich in peptidoglycan was labeled with FITC and incubated with larval hemocytes, and the hemocytes were recovered by centrifugation, washed, and examined by fluorescence microscopy. While intense signals were detectable with control hemocytes, the level of the signal with integrin βν-lacking hemocytes was only marginal (Fig. 4*D*), suggesting the integrin βν-medi-

FIGURE 2. **Requirement of integrin βν for phagocytosis but not humoral response after septic infection with *S. aureus*.** *A*, adult flies of control (w^{1118}) and integrin βν-deficient (*betaInt-nu*^{2}) lines were injected with FITC-labeled wild-type *S. aureus* and analyzed for the level of phagocytosis *in vivo*. Phase contrast and fluorescence views of the same microscopic fields are shown as vertically aligned panels. Parts of fly abdomen containing clusters of fluorescent materials, indicative of phagocytosis by hemocytes, are shown with squares, and their magnified views are exhibited at the *right*. The number of flies that gave the fluorescent clusters similar in size to that shown here is indicated in the panels (the denominator is the total number of flies analyzed). *Scale bars*, 0.5 mm. *B*, larval hemocytes isolated from the indicated fly lines were subjected to immunocytochemistry with anti-integrin βν or control rat serum. Phase contrast and fluorescence views of the same microscopic fields are shown as vertically aligned panels. *Scale bar*, 10 μm. *C*, an assay for phagocytosis *in vitro* was conducted with fluorescence-labeled wild-type *S. aureus*, latex beads, *E. coli*, or *B. subtilis* as targets and larval hemocytes prepared from the indicated fly lines as phagocytes. *D*, adult flies of the indicated lines were injected with wild-type *S. aureus* (*bacteria*) or solvent alone (*PBS*). After 18 h, flies were subjected to quantitative RT-PCR for the amount of drosomycin mRNA, which is shown relative to that in the control experiment (w^{1118} injected with PBS) as 1.

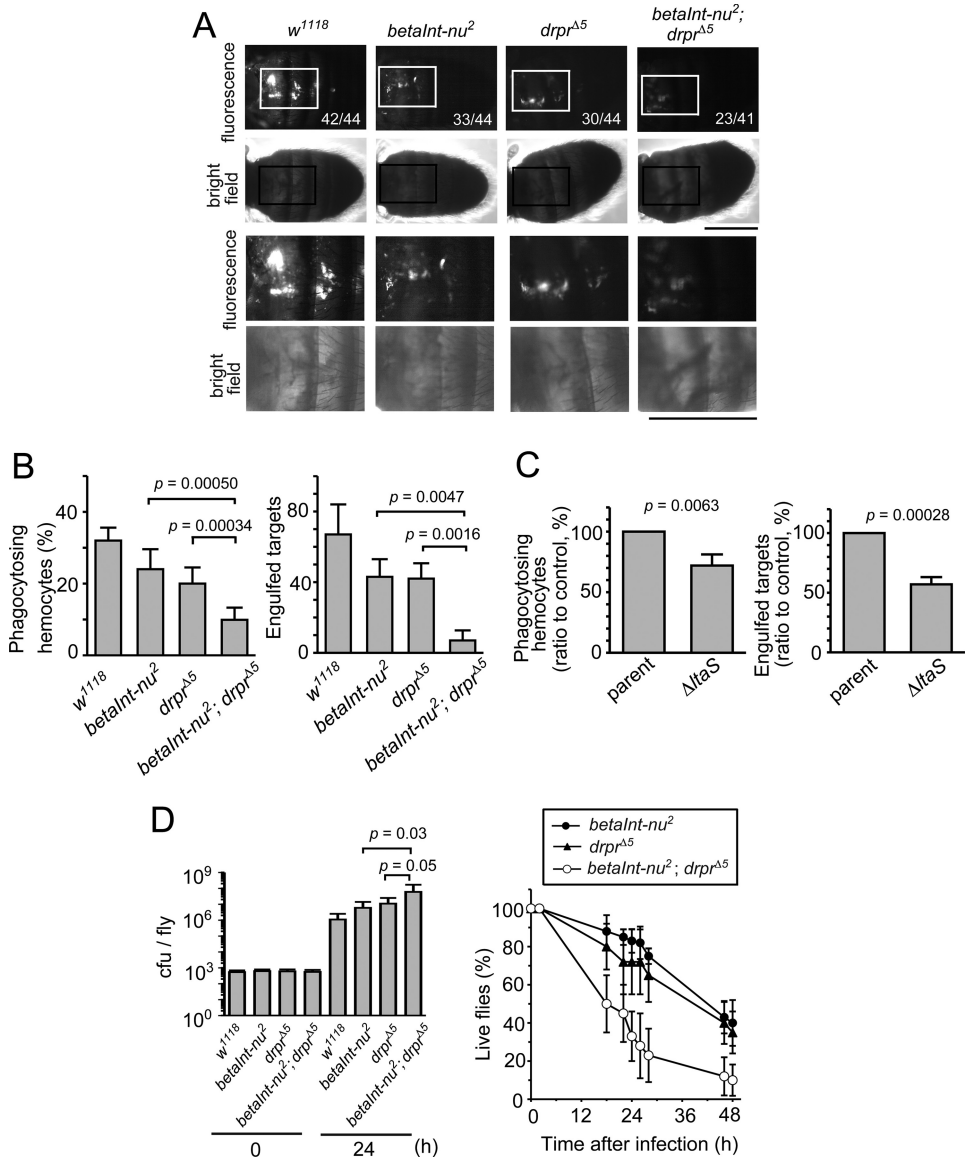
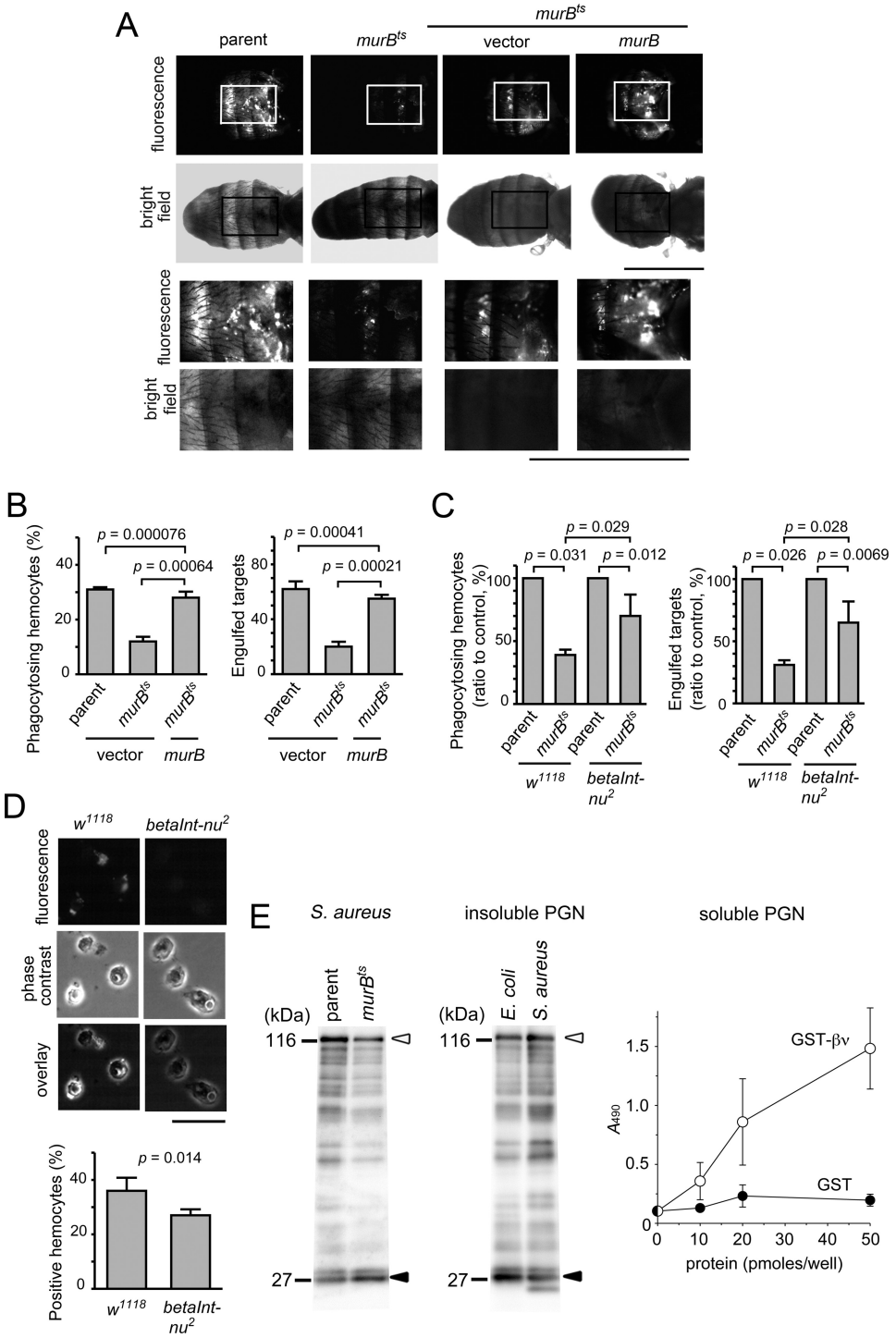


FIGURE 3. **Independent actions of integrin $\beta\nu$ and Draper in defense against *S. aureus*.** *A*, adult flies of control (w^{1118}), integrin $\beta\nu$-deficient (*betaInt-nu*^{2}), and Draper-deficient ($drpr^{\Delta 5}$) lines, and a double mutant for *betaInt-nu* and *drpr* (*betaInt-nu*^{2}*;* $drpr^{\Delta 5}$) were injected with FITC-labeled wild-type *S. aureus* and analyzed for the level of phagocytosis *in vivo*. The data with *betaInt-nu*^{2} and $drpr^{\Delta 5}$ fly lines are a duplicate of those shown in Fig. 2*A*. The micrographs at the *bottom two rows* are magnified views of the area with the *squares* in micrographs at the *top two rows*. The data are presented as in Fig. 2*A*. *Scale bars*, 0.5 mm. *B*, an assay for phagocytosis *in vitro* was conducted with FITC-labeled wild-type *S. aureus* as targets and larval hemocytes prepared from the indicated fly lines as phagocytes. *C*, an assay for phagocytosis *in vitro* was conducted with FITC-labeled wild-type (*parent*) or LtaS-deficient (Δ*ltaS*) *S. aureus* as targets and larval hemocytes prepared from integrin $\beta\nu$-deficient fly lines as phagocytes. The level of phagocytosis is shown relative to that with wild-type bacteria taken as 100. Data from one of three independent experiments with similar results are presented. *D*, adult flies of *betaInt-nu*^{2}, $drpr^{\Delta 5}$, and *betaInt-nu*^{2}*;* $drpr^{\Delta 5}$ lines were injected with wild-type *S. aureus* and analyzed for bacterial growth (*left*) and fly survival (*right*) at the indicated time points. In the *left panel*, cfu values at 24 h relative to those at 0 h were compared between the fly lines. Genotypes of the fly lines analyzed are *w; +;* $drpr^{\Delta 5}$ ($drpr^{\Delta 5}$) and *w; betaInt-nu*^{2}*;* $drpr^{\Delta 5}$ (*betaInt-nu*^{2}*;* $drpr^{\Delta 5}$).

ated binding of peptidoglycan to hemocytes. We next examined the binding of integrin $\beta\nu$ to *S. aureus* strains. Bacteria, parental, or *murB* mutant strain, were incubated with a mixture of GST-fused recombinant protein corresponding to the extracellular region of integrin $\beta\nu$ and GST alone at an equal molar ratio, precipitated by centrifugation, lysed, and analyzed by Western blotting for the presence of the GST proteins. The signal corresponding to GST-fused integrin $\beta\nu$ was more intense than that of GST alone using the parental *S. aureus* strain, but this was not the case in the experiment using the *murB* mutant (*left panel* in Fig. 4*E*). To directly analyze the binding of integrin $\beta\nu$ to peptidoglycan, insoluble preparations of peptidoglycan obtained from *S. aureus* (parental strain) and *E. coli* were incubated with a mixture of GST-fused integrin $\beta\nu$ and GST, precipitated, and subjected to Western blotting with anti-GST antibody. We found that more GST-fused integrin $\beta\nu$ was recovered than GST alone using *S. aureus* peptidoglycan while recovery of the two GST proteins did not significantly differ using peptidoglycan prepared from *E. coli* (*middle panel* in Fig. 4*E*). Finally, we analyzed the binding of integrin $\beta\nu$ to a soluble preparation of peptidoglycan in a solid-phase assay. GST-fused integrin $\beta\nu$ showed dose-dependent adhesion to

culture dishes coated with *S. aureus* peptidoglycan, but GST alone did not give appreciable binding (*right panel* in Fig. 4*E*). We were unable to examine the binding of integrin $\beta\nu$ to a soluble preparation of *E. coli* peptidoglycan that caused extremely high background in this assay. These results collectively indicated a physical association between integrin $\beta\nu$ and peptidoglycan of *S. aureus*.

Involvement of Rac but Not Elmo in Integrin βν-mediated Phagocytosis of S. aureus—We next addressed an event located downstream of integrin $\beta\nu$ for the induction of the phagocytosis of *S. aureus*. There are two partly overlapping pathways for the induction of the phagocytosis of apoptotic cells in *C. elegans*, namely, CED-1-CED-6/CED-7-CED-10 and CED-2-CED-5-CED-12-CED-10, which are presumably conserved among species (41, 42). Draper appears to be located at the furthest point upstream of the former pathway in the phagocytosis of apoptotic cells (43) and degenerated neural axons (44). This suggests the involvement of the latter pathway in the integrin $\beta\nu$-mediated phagocytosis of *S. aureus* although this is not clear in the integrin $\beta\nu$-mediated phagocytosis of apoptotic



cells (31). *Drosophila* counterparts of *C. elegans* CED-12 and CED-10 are Elmo and Rac, respectively (41, 42). There are three RacGTPases; namely, Rac1, Rac2, and Mig2-like, besides Rho and Cdc42 that together constitute the *Drosophila* RhoGTPase family of protein. We examined the involvement of Rac1 and Rac2 in the phagocytosis of *S. aureus* because previous reports showed that these two GTPases are necessary for the cellular immune response by *Drosophila* hemocytes: Rac2 is required for the phagocytosis of bacteria (45), and both Rac1 and Rac2 are required for the encapsulation of wasp eggs (46, 47).

We first examined the involvement of Elmo, a *Drosophila* homologue of CED-12 and a component of a guanine nucleotide exchange factor specific for the small G protein Rac (48). However, larval hemocytes lacking the expression of Elmo showed phagocytic activity comparable to those prepared from control flies (Fig. 5*A*), suggesting that Elmo is dispensable. In contrast, hemocytes isolated from flies heterozygous for mutant alleles of both *betaInt-nu* and *Rac* showed a decreased level of phagocytosis compared with those from a heterozygote of either mutation (Fig. 5*B*), indicating a genetic interaction between the two genes. These results suggested that Rac1/Rac2 is contained in a signaling pathway located downstream of integrin $\beta\nu$ for the induction of the phagocytosis of *S. aureus*.

DISCUSSION

In the present study, we found that *betaInt-nu* coding for $\beta\nu$, a β subunit of *Drosophila* integrins, participates in the phagocytosis of Gram-positive *S. aureus* by hemocytes. We previously reported that Draper, another membrane-transverse protein of *Drosophila* hemocytes, is involved in the phagocytosis of the same bacterium (26). Here we showed that Draper and integrin $\beta\nu$ cooperate in the defense against *S. aureus* to confer flies prolonged survival after a septic infection with the bacterium. The two receptors appeared to recognize distinct cell wall components as ligands for phagocytosis: Draper binds to lipoteichoic acid (26) while integrin $\beta\nu$ does peptidoglycan. This suggests the presence of a dual recognition system by hemocytes for the efficient removal of *S. aureus* by phagocytosis. Signaling pathways located downstream of the receptors remain to be clarified, but they both seemed to require Rac, a small G protein involved in the phagocytosis of apoptotic cells (41, 42), suggesting that the two pathways overlap at least partly. Integrin $\beta\nu$ seemed not required for the phagocytosis of *E. coli* and *B. subtilis* by larval hemocytes. The structure of peptidoglycan

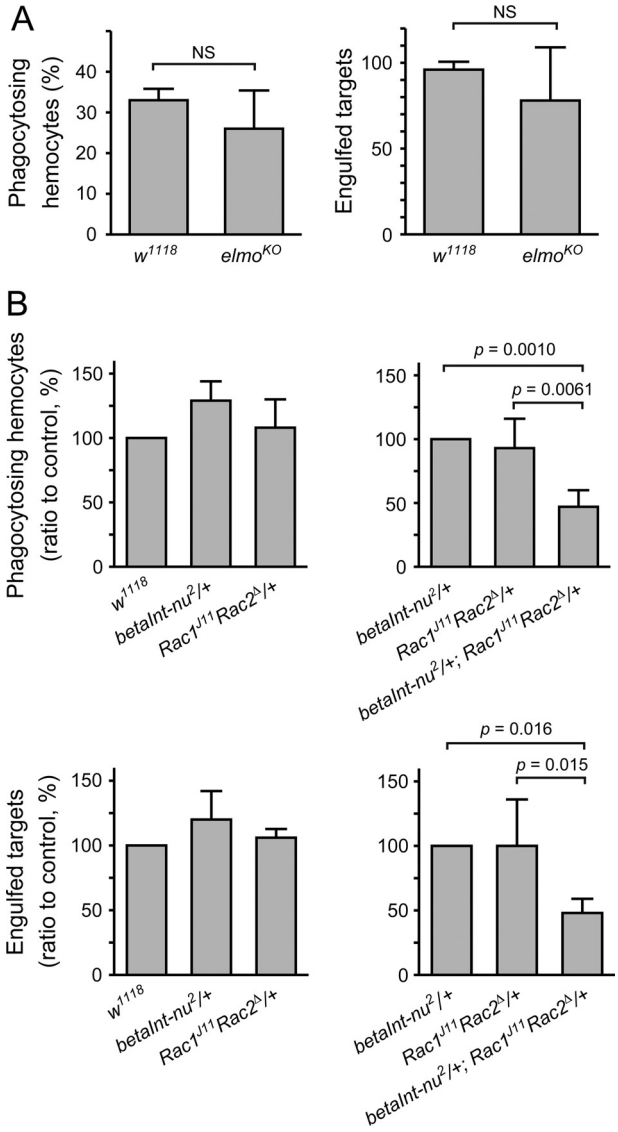


FIGURE 5. **Genetic interaction between *betaInt-nu* and *rac1*/*rac2* in phagocytosis of *S. aureus*.** An assay for phagocytosis *in vitro* was conducted with FITC-labeled wild-type *S. aureus* as targets and hemocytes prepared from larvae of control flies (w^{1118}) (*A*), *elmo* null flies ($elmo^{KO}$) (*A*), flies heterozygous for *betaInt-nu*^{2} (*betaInt-nu*^{2}/+) (*B*), flies heterozygous for *rac1* and *rac2* ($Rac1^{J11}$ $Rac2^{\Delta}$/+) (*B*), or flies trans-heterozygous for *betaInt-nu*^{2}, *rac1*, and *rac2* (*betaInt-nu*^{2}/$Rac1^{J11}$ $Rac2^{\Delta}$) (*B*) as phagocytes. Genotypes of the fly lines used are $elmo^{KO}$*FRT40, 42*/$elmo^{KO}$*FRT40, 42* ($elmo^{KO}$) and y^1 w^*; $Rac1^{J11}$ $Rac2^{\Delta}$ *FRT2A/TM6B,* Tb^+ ($Rac1^{J11}$ $Rac2^{\Delta}$).

FIGURE 4. **Identification of *S. aureus murB* required for integrin $\beta\nu$-mediated phagocytosis.** *A*, adult flies of the control (w^{1118}) line were injected with 5-carboxyfluorescein-labeled wild-type (*parent*) or *murB* mutant ($murB^{ts}$) *S. aureus* and analyzed for the level of phagocytosis *in vivo*. The mutant strain was also analyzed for susceptibility to phagocytosis with (*murB*) or without (*vector*) complementation of the wild-type gene. The data are aligned and presented as in Fig. 3*A*. *Scale bars*, 0.5 mm. *B*, an assay for phagocytosis *in vitro* was conducted with FITC-labeled *S. aureus* as targets and larval hemocytes prepared from control (w^{1118}) lines as phagocytes. The bacteria used are wild-type (*parent*) and *murB* mutant ($murB^{ts}$) strains with (*murB*) and without (*vector*) complementation of the wild-type gene. *C*, an assay for phagocytosis *in vitro* was conducted with FITC-labeled wild-type (*parent*) and *murB* mutant ($murB^{ts}$) *S. aureus* as targets and larval hemocytes prepared from control (w^{1118}) and integrin $\beta\nu$-deficient (*betaInt-nu*^{2}) lines as phagocytes. The extent of phagocytosis is shown relative to that with wild-type bacteria taken as 100. *D*, larval hemocytes prepared from the indicated fly lines were incubated with FITC-labeled peptidoglycan prepared from wild-type *S. aureus*, washed, and examined by fluorescence microscopy. *Left*, fluorescence, bright field, and overlaid views of the same microscopic fields are shown as vertically aligned panels. *Right*, number of hemocytes bound by peptidoglycan was determined and is shown relative to the total number of hemocytes analyzed. *Scale bar*, 50 μm. *E*, binding of the extracellular region of integrin $\beta\nu$ to *S. aureus* strains (*left*), insoluble peptidoglycan (*PGN*) (*middle*), and soluble peptidoglycan (*right*) was examined. A mixture of GST-fused integrin $\beta\nu$ and GST at an equal molar ratio was incubated with bacteria (*left*) or insoluble peptidoglycan (*middle*) and centrifuged, and the resulting precipitates were subjected to Western blotting with anti-GST antibody. The *open* and *closed arrowheads* indicate the positions of GST-fused integrin $\beta\nu$ and GST, respectively. In the *right panel*, GST-fused integrin $\beta\nu$ (*GST-*$\beta\nu$) (*open circles*) and GST (*closed circles*) were incubated in culture dishes coated with soluble peptidoglycan prepared from parental *S. aureus*, and the amount of GST proteins attached to dishes was immunochemically determined. Data from one of three independent experiments that yielded similar results are presented.

of *S. aureus* differs from that of these two bacteria species particularly in the composition of peptide, suggesting that a peptide portion of peptidoglycan is involved, either directly or indirectly, in the recognition by integrin $\beta\nu$. In fact, our data suggested that integrin $\beta\nu$ does not effectively recognize peptidoglycan of *E. coli*.

Integrins exist as heterodimers of two trans-membrane proteins named α and β (49–51). There are five α (αPS1, 2, 3, 4, and 5) and two β (βPS and $\beta\nu$) subunits for *Drosophila* integrins (52, 53). It is unknown at present which of the five α subunits forms a heterodimer with $\beta\nu$ and acts as a receptor for the phagocytosis of *S. aureus*. The other β subunit of *Drosophila* integrin, βPS, has been shown to be required for the encapsulation by lamellocytes of larvae of the wasp *Leptopilina boulardi* (54), and the small G protein Rac1 is located downstream of βPS for the induction of encapsulation (55). Rac is thus likely responsible for the induction of a change of cell shape and cell motility so that hemocytes accomplish cellular immune reactions. Integrins of other insect species have been reportedly involved in the phagocytosis of bacteria: larval hemocytes of *Ceratitis capitata*, a Mediterranean fruit fly, seem to engulf *E. coli in vitro* in a manner mediated by βPS (56, 57); and the β subunit BINT2 of the mosquito *Anopheles gambiae* is required for the phagocytosis of *E. coli* but not *S. aureus* both *in vivo* (58) and *in vitro* (59). These findings indicate that insect integrins in general play roles in the defense against invasive microorganisms.

The three receptors for the phagocytosis of bacteria in *Drosophila*, namely, Croquemort (27, 28), Draper (29, 30), and integrin $\beta\nu$ (31), were originally found as receptors responsible for the phagocytic elimination of apoptotic cells. Another example of the participation of a receptor in the phagocytosis of both apoptotic cells and bacteria comes from mammals. There is a mammalian receptor for phagocytosis, named brain angiogenesis inhibitor 1, which is required for the phagocytic elimination of dying cells in a manner mediated by the recognition of phosphatidylserine exposed at the surface of target cells (60). This receptor was recently reported to be responsible for the phagocytosis of *Salmonella typhimurium*, a Gram-negative bacterium, by a murine macrophage cell line, recognizing the cell wall component LPS (36). It is therefore possible that some receptors for phagocytosis target both altered self-tissues and invading microorganisms as part of innate immune responses. In any case, it remains to be solved how such receptors recognize apparently distinct structures present at the surface of apoptotic cells and bacteria. Our results indicate that integrin $\beta\nu$ recognizes peptidoglycan at the surface of *S. aureus*. Other phagocytosis receptors of *Drosophila* such as peptidoglycan recognition protein LC (19) and Eater (24) have been suggested to use peptidoglycan as a ligand for the phagocytosis of bacteria. Taken together, there might exist a multiple recognition system for the effective removal of invading bacteria by phagocytosis in *Drosophila*; that is, multiple receptors with distinct ligand-specificity in the phagocytosis of a single bacterium as well as multiple receptors with the same ligand-specificity in the phagocytosis of different bacteria species containing the same structure.

Acknowledgments—We thank Kenji Kurokawa, Yasuhiko Matsumoto, and Motoyuki Sugai for the bacterial strains, and Nicholas Brown, Marc Freeman, Pernille Rørth, Bloomington Drosophila Stock Centre, and Kyorin-Fly for the fly lines. Yukichika Tabuchi, Yumi Hashimoto, Yoichi Osada, and Kazuki Takeuchi are thanked for their contribution to this study at the initial stage.

REFERENCES

1. Aderem, A., and Underhill, D. M. (1999) Mechanism of phagocytosis in macrophages. *Annu. Rev. Immunol.* **17,** 593–623
2. Stuart, L. M., and Ezekowitz, R. A. (2005) Phagocytosis: elegant complexity. *Immunity* **22,** 539–550
3. Braun, A., Hoffmann, J. A., and Meister, M. (1998) Analysis of the *Drosophila* host defense in domino mutant larvae, which are devoid of hemocytes. *Proc. Natl. Acad. Sci. U.S.A.* **95,** 14337–14342
4. Elrod-Erickson, M., Mishra, S., and Schneider, D. (2000) Interactions between the cellular and humoral immune responses in *Drosophila. Curr. Biol.* **10,** 781–784
5. Haine, E. R., Moret, Y., Siva-Jothy, M. T., and Rolff, J. (2008) Antimicrobial defense and persistent infection in insects. *Science* **322,** 1257–1259
6. Nehme, N. T., Quintin, J., Cho, J. H., Lee, J., Lafarge, M.-C., Kocks, C., and Ferrandon, D. (2011) Relative roles of the cellular and humoral responses in the *Drosophila* host defense against three Gram-positive bacterial infections. *PLoS. ONE.* **6,** e14743
7. Basset, A., Khush, R. S., Braun, A., Gardan, L., Boccard, F., Hoffmann, J. A., and Lemaitre, B. (2000) The phytopathogenic bacteria *Erwinia carotovora* infects *Drosophila* and activates an immune response. *Proc. Natl. Acad. Sci. U.S.A.* **97,** 3376–3381
8. Foley, E., and O'Farrell, P. H. (2003) Nitric oxide contributes to induction of innate immune responses to gram-negative bacteria in *Drosophila. Genes Dev.* **17,** 115–125
9. Brennan, C. A., Delaney, J. R., Schneider, D. S., and Anderson, K. V. (2007) Psidin is required in *Drosophila* blood cells for both phagocytic degradation and immune activation of the fat body. *Curr. Biol.* **17,** 67–72
10. Nehme, N. T., Liégeois, S., Kele, B., Giammarinaro, P., Pradel, E., Hoffmann, J. A., Ewbank, J. J., and Ferrandon, D. (2007) A model of bacterial intestinal infections in *Drosophila melanogaster. PLoS. Pathog.* **3,** e173
11. Charroux, B., and Royet, J. (2009) Elimination of plasmatocytes by targeted apoptosis reveals their role in multiple aspects of the *Drosophila* immune response. *Proc. Natl. Acad. Sci. U.S.A.* **106,** 9797–9802
12. Defaye, A., Evans, I., Crozatier, M., Wood, W., Lemaitre, B., and Leulier, F. (2009) Genetic ablation of *Drosophila* phagocytes reveals their contribution to both development and resistance to bacterial infection. *J. Innate Immun.* **1,** 322–334
13. Mylonakis, E., and Aballay, A. (2005) Worms and flies as genetically tractable animal models to study host-pathogen interactions. *Infect. Immun.* **73,** 3833–3841
14. Khush, R. S., and Lemaitre, B. (2000) Genes that fight infection: what the *Drosophila* genome says about animal immunity. *Trends Genet.* **16,** 442–449
15. Lemaitre, B., and Hoffmann, J. (2007) The host defense of *Drosophila melanogaster. Annu. Rev. Immunol.* **25,** 697–743
16. Strand, M. R. (2008) The insect cellular immune response. *Insect Sci.* **15,** 1–14
17. Fauvarque, M. O., and Williams, M. J. (2011) Drosophila cellular immunity: a story of migration and adhesion. *J. Cell Sci.* **124,** 1373–1382
18. Stuart, L. M., and Ezekowitz, R. A. (2008) Phagocytosis and comparative innate immunity: learning on the fly. *Nat. Rev. Immunol.* **8,** 131–141
19. Rämet, M., Manfruelli, P., Pearson, A., Mathey-Prevot, B., and Ezekowitz, R. A. (2002) Functional genomic analysis of phagocytosis and identification of a *Drosophila* receptor for *E. coli. Nature* **416,** 644–648
20. Philips, J. A., Rubin, E. J., and Perrimon, N. (2005) *Drosophila* RNAi screen reveals CD36 family member required for *Mycobacterial* infection. *Science* **309,** 1251–1253
21. Rämet, M., Pearson, A., Manfruelli, P., Li, X., Koziel, H., Göbel, V., Chung, E., Krieger, M., and Ezekowitz, R. A. (2001) *Drosophila* scavenger receptor

CI is a pattern recognition receptor for bacteria. *Immunity* **15,** 1027–1038
22. Stuart, L. M., Deng, J., Silver, J. M., Takahashi, K., Tseng, A. A., Hennessy, E. J., Ezekowitz, R. A., and Moore, K. J. (2005) Response to *Staphylococcus aureus* required CD36-mediated phagocytosis triggered by the COOH-terminal cytoplasmic domain. *J. Cell Biol.* **170,** 477–485
23. Kocks, C., Cho, J. H., Nehme, N., Ulvila, J., Pearson, A. M., Meister, M., Strom, C., Conto, S. L., Hetru, C., Stuart, L. M., Stehle, T., Hoffmann, J. A., Reichhart, J.-M., Ferrandon, D., Rämet, M., and Ezekowitz, R. A. B. (2005) Eater, a transmembrane protein mediating phagocytosis of bacterial pathogens in *Drosophila. Cell* **123,** 335–346
24. Chung, Y. S., and Kocks, C. (2011) Recognition of pathogenic microbes by the *Drosophila* phagocytic pattern recognition receptor Eater. *J. Biol. Chem.* **286,** 26524–26532
25. Kurucz, E., Márkus, R., Zsámboki, J., Folkl-Medzihradszky, K., Darula, Z., Vilmos, P., Udvardy, A., Krausz, I., Lukacsovich, T., Gateff, E., Zettervall, C. J., Hultmark, D., and Andó, I. (2007) Nimrod, a putative phagocytosis receptor with EGF repeats in *Drosophila* plasmatocytes. *Curr. Biol.* **17,** 649–654
26. Hashimoto, Y., Tabuchi, Y., Sakurai, K., Kutsuna, M., Kurokawa, K., Awasaki, T., Sekimizu, K., Nakanishi, Y., and Shiratsuchi, A. (2009) Identification of lipoteichoic acid as a ligand for Draper in the phagocytosis of *Staphylococcus aureus* by *Drosophila* hemocytes. *J. Immunol.* **183,** 7451–7460
27. Franc, N. C., Dimarcq, J. L., Lagueux, M., Hoffmann, J., and Ezekowitz, R. A. (1996) Croquemort, a novel *Drosophila* hemocyte/macrophage receptor that recognizes apoptotic cells. *Immunity* **4,** 431–443
28. Franc, N. C., Heitzler, P., Ezekowitz, R. A. B., and White, K. (1999) Requirement for Croquemort in phagocytosis of apoptotic cells in *Drosophila. Science* **284,** 1991–1994
29. Freeman, M. R., Delrow, J., Kim, J., Johnson, E., and Doe, C. Q. (2003) Unwrapping glial biology: Gcm target genes regulating glial development, diversification, and function. *Neuron* **38,** 567–580
30. Manaka, J., Kuraishi, T., Shiratsuchi, A., Nakai, Y., Higashida, H., Henson, P., and Nakanishi, Y. (2004) Draper-mediated and phosphatidylserine-independent phagocytosis of apoptotic cells by *Drosophila* hemocytes/macrophages. *J. Biol. Chem.* **279,** 48466–48476
31. Nagaosa, K., Okada, R., Nonaka, S., Takeuchi, K., Fujita, Y., Miyasaka, T., Manaka, J., Ando, I., and Nakanishi, Y. (2011) Integrin $\beta\nu$-mediated phagocytosis of apoptotic cells in *Drosophila* embryos. *J. Biol. Chem.* **286,** 25770–25777
32. Oku, Y., Kurokawa, K., Matsuo, M., Yamada, S., Lee, B. L., and Sekimizu, K. (2009) Pleiotropic roles of polyglycerolphosphate synthase of lipoteichoic acid in growth of *Staphylococcus aureus* cells. *J. Bacteriol.* **191,** 141–151
33. Nishida, S., Kurokawa, K., Matsuo, M., Sakamoto, K., Ueno, K., Kita, K., and Sekimizu, K. (2006) Identification and characterization of amino acid residues essential for the active site of UDP-*N*-acetylenolpyruvylglucosamine reductase (MurB) from *Staphylococcus aureus. J. Biol. Chem.* **281,** 1714–1724
34. Devenport, D., and Brown, N. H. (2004) Morphogenesis in the absence of integrins: mutation of both *Drosophila* β subunits prevents midgut migration. *Development* **131,** 5405–5415
35. Bianco, A., Poukkula, M., Cliffe, A., Mathieu, J., Luque, C. M., Fulga, T. A., and Rørth, P. (2007) Two distinct modes of guidance signaling during collective migration of border cells. *Nature* **448,** 362–365
36. Das, S., Owen, K. A., Ly, K. T., Park, D., Black, S. G., Wilson, J. M., Sifri, C. D., Ravichandran, K. S., Ernst, P. B., and Casanova, J. E. (2011) Brain angiogenesis inhibitor 1 (BAI1) is a pattern recognition receptor that mediates macrophage binding and engulfment of Gram-negative bacteria. *Proc. Natl. Acad. Sci. U.S.A.* **108,** 2136–2141
37. Avet-Rochex, A., Bergeret, E., Attree, I., Meister, M., and Fauvarque, M.-O. (2005) Suppression of *Drosophila* cellular immunity by directed expression of the ExoS toxin GAP domain of *Pseudomonas aeruginosa. Cell. Microbiol.* **7,** 799–810
38. Ha, H. M., Oh, C. T., Bae, Y. S., and Lee, W. J. (2005) A direct role for dual oxidase in *Drosophila* gut immunity. *Science* **310,** 847–850
39. Park, J. W., Kim, C. H., Kim, J. H., Je, B. R., Roh, K. B., Kim, S. J., Lee, H. H., Ryu, J. H., Lim, J. H., Oh, B. H., Lee, W. J., Ha, N. C., and Lee, B. L. (2007) Clustering of peptidoglycan recognition protein-SA is required for sensing lysine-type peptidoglycan in insects. *Proc. Natl. Acad. Sci. U.S.A.* **104,** 6602–6607
40. Yu, Y., Park, J. W., Kwon, H. M., Hwang, H. O., Jang, I. H., Masuda, A., Kurokawa, K., Nakayama, H., Lee, W. J., Dohmae, N., Zhang, J., and Lee, B. L. (2010) Diversity of innate immune recognition mechanism for bacterial polymeric *meso*-diaminopimelic acid-type peptidoglycan. *J. Biol. Chem.* **285,** 32937–32945
41. Lettre, G., and Hengartner, M. O. (2006) Developmental apoptosis in *C. elegans*: a complex CEDnario. *Nat. Rev. Mol. Cell Biol.* **7,** 97–108
42. Kinchen, J. M., and Ravichandran, K. S. (2007) Journey to the grave: signaling events regulating removal of apoptotic cells. *J. Cell Sci.* **120,** 2143–2149
43. Kuraishi, T., Nakagawa, Y., Nagaosa, K., Hashimoto, Y., Ishimoto, T., Moki, T., Fujita, Y., Nakayama, H., Dohmae, N., Shiratsuchi, A., Yamamoto, N., Ueda, K., Yamaguchi, M., Awasaki, T., and Nakanishi, Y. (2009) Pretaporter, a *Drosophila* protein serving as a ligand for Draper in the phagocytosis of apoptotic cells. *EMBO J.* **28,** 3868–3878
44. Awasaki, T., Tatsumi, R., Takahashi, K., Arai, K., Nakanishi, Y., Ueda, R., and Ito, K. (2006) Essential role of the apoptotic cell engulfment genes *draper* and *ced-6* in programmed axon pruning during *Drosophila* metamorphosis. *Neuron* **50,** 855–867
45. Avet-Rochex, A., Perrin, J., Bergeret, E., and Fauvarque, M. O. (2007) Rac2 is a major actor of *Drosophila* resistance to *Pseudomonas aeruginosa* acting in phagocytic cells. *Genes Cells* **12,** 1193–1204
46. Williams, M. J., Ando, I., and Hultmark, D. (2005) *Drosophila* melanogaster Rac2 is necessary for a proper cellular immune response. *Genes Cells* **10,** 813–823
47. Williams, M. J., Wiklund, M. L., Wikman, S., and Hultmark, D. (2006) Rac1 signalling in the *Drosophila* larval cellular immune response. *J. Cell Sci.* **119,** 2015–2024
48. Côté, J. F., and Vuori, K. (2007) GEF what? Dock180 and related proteins help Rac to polarize cells in new ways. *Trends Cell Biol.* **17,** 383–393
49. Giancotti, F. G., and Ruoslahti, E. (1999) Integrin signaling. *Science* **285,** 1028–1032
50. Hynes, R. O. (2002) Integrins: bidirectional, allosteric signaling machines. *Cell* **110,** 673–687
51. Caswell, P. T., Vadrevu, S., and Norman, J. C. (2009) Integrins: masters and slaves of endocytic transport. *Nat. Rev. Mol. Cell Biol.* **10,** 843–853
52. Brown, N. H., Gregory, S. L., and Martin-Bermudo, M. D. (2000) Integrins as mediators of morphogenesis in *Drosophila. Dev. Biol.* **223,** 1–16
53. Brower, D. L. (2003) Platelets with wings: the maturation of *Drosophila* integrin biology. *Curr. Opin. Cell Biol.* **15,** 607–613
54. Irving, P., Ubeda, J. M., Doucet, D., Troxler, L., Lagueux, M., Zachary, D., Hoffmann, J. A., Hetru, C., and Meister, M. (2005) New insights into *Drosophila* larval haemocyte functions through genome-wide analysis. *Cell. Microbiol.* **7,** 335–350
55. Xavier, M. J., and Williams, M. J. (2011) The Rho-family GTPase Rac1 regulates integrin localization in *Drosophila* immunosurveillance cells. *PLoS. ONE.* **6,** e19504
56. Foukas, L. C., Katsoulas, H. L., Paraskevopoulou, N., Metheniti, A., Lambropoulou, M., and Marmaras, V. J. (1998) Phagocytosis of *Escherichia coli* by insect hemocytes requires both activation of the Ras/mitogen-activated protein kinase signal transduction pathway for attachment and β3 integrin for internalization. *J. Biol. Chem.* **273,** 14813–14818
57. Mamali, I., Lamprou, I., Karagiannis, F., Karakantza, M., Lampropoulou, M., and Marmaras, V. J. (2009) A β integrin subunit regulates bacterial phagocytosis in medfly haemocytes. *Dev. Comp. Immunol.* **33,** 858–866
58. Moita, L. F., Wang-Sattler, R., Michel, K., Zimmermann, T., Blandin, S., Levashina, E. A., and Kafatos, F. C. (2005) *In vitro* identification of novel regulators and conserved pathways of phagocytosis in *A. gambiae. Immunity* **23,** 65–73
59. Moita, L. F., Vriend, G., Mahairaki, V., Louis, C., and Kafatos, F. C. (2006) Integrins of *Anopheles gambiae* and a putative role of a new β integrin, BINT2, in phagocytosis of *E. coli. Insect Biochem. Mol. Biol.* **36,** 282–290
60. Park, D., Tosello-Trampont, A. C., Elliott, M. R., Lu, M., Haney, L. B., Ma, Z., Klibanov, A. L., Mandell, J. W., and Ravichandran, K. S. (2007) BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. *Nature* **450,** 430–434