The FASEB Journal express article 10.1096/fj.02-1139fje. Published online June 17, 2003.

T cells respond to heat shock protein 60 via TLR2: activation of adhesion and inhibition of chemokine receptors

Alexandra Zanin-Zhorov, Gabriel Nussbaum, Susanne Franitza, Irun R. Cohen, and Ofer Lider

The Department of Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel

Corresponding author: Irun R. Cohen, Department of Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel. E-mail: irun.cohen@weizmann.ac.il

ABSTRACT

Soluble 60 kDa heat shock protein (HSP60) activates macrophages via TLR4. We now report that soluble HSP60 activates T cells via the innate receptor TLR2. HSP60 activated T cell adhesion to fibronectin to a degree similar to other activators: IL-2, SDF-1α, and RANTES. T cell type and state of activation was important; nonactivated $CD45RA^+$ and IL-2-activated $CD45RO^+$ T cells responded optimally (1 h) at low concentrations (0.1-1 ng/ml), but nonactivated $CD45RO^+$ T cells required higher concentrations (~1 μg/ml) of HSP60. T cell HSP60 signaling was inhibited specifically by monoclonal antibodies (mAb) to TLR2 but not by a mAb to TLR4. Indeed, T cells from mice with mutated TLR4 could still respond to HSP60, whereas Chinese hamster T cells with mutated TLR2 did not respond. The human T cell response to soluble HSP60 depended on phosphatidylinositol 3-kinase and protein kinase C signaling and involved the phosphorylation of Pyk-2. Soluble HSP60 also inhibited actin polymerization and T cell chemotaxis through extracellular matrix-like gels toward the chemokines SDF-1α (CXCL12) or ELC (CCL19). Exposure to HSP60 for longer times (18 h) down-regulated chemokine receptor expression: CXCR4 and CCR7. These results suggest that soluble HSP60, through TLR2-dependent interactions, can regulate T cell behavior in inflammation.

Key words: chemotaxis • extracellular matrix • HSP • inflammation • SDF-1α

The mammalian 60 kDa heat shock protein (HSP60) is a molecule of many facets. In addition to serving as a chaperone, HSP60 is expressed by cells exposed to stress (1–3) or immune activation (4–8), is present in the blood during inflammation (8–13), and has been found to be a target of autoantibodies and autoimmune T cells in healthy individuals and, to a greater extent, in those suffering from autoimmune diseases (14–16). Recently, HSP60 has been discovered to activate macrophages by way of the innate toll-like receptor 4 (TLR4) to induce enhanced production of NO and IL-12, IL-15, TNF-α, IL-6, and other Th1-type cytokines (17–19). Thus HSP60 would seem to be an important factor in inflammation generally. The present study was done to test whether HSP60 might also exert a direct, innate effect on T cell physiology.

T cells move to sites of inflammation using a variety receptors (20). After their passage through the blood-vessel endothelium, migrating T cells interact with components of the extracellular

matrix (ECM), particularly with glycoproteins such as collagen, laminin and fibronectin (FN; refs 20, 21). The β1-integrin molecules of T cells enable them to adhere to and migrate through the ECM, a process that is affected by a variety of cytokines, chemokines, and acute phase proteins situated within the context of the ECM (21). Unlike antigen recognition, the physiology of T cell migration and adhesion is accomplished without the mediation of APC. Herein, we tested whether soluble HSP60 might affect the ECM-associated adhesion and chemotactic migration of T cells and the expression and function of T cell chemokine receptors.

MATERIAL AND METHODS

Reagents

The following reagents and chemicals were purchased from the sources indicated: RPMI-1640 (Gibco BRL, Paisley, UK), FCS, HEPES buffer, antibiotics, sodium pyruvate (Kibbutz Beit-Haemek; Israel); collagen type I (Cellagen; ICN Pharmaceuticals, Costa-mesa, CA); FN (Chemicon, Temecula, CA); laminin (Sigma, Rehovot, Israel); recombinant human IL-2 (Chiron, Amsterdam, The Netherlands); SDF-1α, ELC, RANTES, VEGF, βFGF (R&D Systems; Minneapolis, MN); PMA (Sigma, Rehovot, Israel). Monoclonal antibodies (mAb) directed against human β1 integrins (CD29, clone 3SS), and their α-chain subunits, α_2, α_3, α_4, and α_5 (clones AK7, 17C6, HP2/1, and JBSS, respectively), and anti-human CD44 (clone 5035-41.1D), anti-human LFA-1 (clone DF1524), and anti-human L-selectin (clone DREG-56) were obtained from Serotec (Oxford, UK); mAb directed against toll-like receptor (TLR)-2 and TLR4 were obtained from eBioscience (San Diego, CA); mAb (IgM) anti-human recombinant HSP60, designated clone P5, was kindly provided by our colleague, Francisco Quintana; anti-phosphorylated Pyk2 (clone py881) was obtained from Biosource (Camarillo, CA); anti-total Pyk2 (clone N-19) from Santa-Cruz Biotech (Santa Cruz, CA); and anti-human CXCR4 (clone 44708.111) and anti-human CCR7 (clone 6B3) were purchased from R&D Systems and MBL (Nagoya, Japan), respectively. Recombinant HSP60 was purchased from StressGen Biotechnologies (Victoria, BC, Canada); GroEL (bacterial HSP 60), polymyxin B (PMB), and LPS of *E. Coli* (O127:B8) were from Sigma; and purified recombinant HSP65 of *Mycobacterium tuberculosis* (MT-HSP65) was kindly provided by Dr. R. Van der Zee (Faculty of Veterinary Medicine, Utrecht, The Netherlands). The HSP60 used in this study was tested for bacterial endotoxin using the kinetic-Turbidimetric LAL test method (Biological Industries Co., Kibutz Beit-Haemek, Israel) and found to contain <0.001 EU/ml (<0.1 pg/ml). $Na_2{}^{51}[Cr]O_4$ was purchased from Amersham Pharmacia Biotech (Little Chalfont, UK).

Human T cells

T cells were purified from healthy human donor peripheral blood. The cells were isolated on Ficoll-Histopaque prepacked columns and washed. B cells and monocytes were depleted as described previously (22). Next, $CD3^+$ T cells were isolated by negative selection with a mouse anti-human antibody cocktail (Pan T cell kit; Miltenyi Biotec; Germany) containing mAb against CD11b, CD16, CD19, CD36, and CD56. The labeled cells were then passed through separation columns (MidiMACS columns, Miltenyi Biotec). In a second round of purification, $CD3^+$ T cells were labeled for negative selection with magnetically coupled mAb against $CD45RA^+$ and $CD45RO^+$ (Miltenyi Biotec). The purified cells obtained (usually >97% $CD45RO^+$ or $CD45RA^+$ T cells) were cultured in RPMI containing antibiotics and 10% heat-inactivated FCS.

Mouse and Chinese hamster thymocytes

Cells were obtained from the thymuses of C3HeB/FeJ and C3H/HeJ mice or Chinese hamsters (females, 6 wk old). After the preparation of single cell suspensions, the cells were untreated or treated with HSP60 as described.

T cell adhesion and migration assays.

Analysis of T cell adhesion to ECM components was determined as described previously (22). Briefly, flat-bottom microtiter well plates were precoated with FN, laminin, or collagen type I (10 μg/ml), and the remaining binding sites were blocked with 1% BSA. Next, ^{51}Cr-labeled T cells were resuspended in RPMI medium supplemented with 1% HEPES buffer and 0.1% BSA (adhesion medium), preincubated (30 min, 37°C, 7% CO_2 in a humidified atmosphere) with HSP60, GroEL MT-HSP65, or LPS, and the cells were then added to the wells. The plates containing the test materials were further incubated (30 min, 37°C in a 7% CO_2 humidified atmosphere) and then gently washed. The adherent cells were lysed (1 M NaOH, 0.1% Triton X-100 in H_2O), removed, and counted using a γ-counter (Packard, Downers Grove, IL). The results are expressed as the mean (±SD) percentage of bound T cells from quadruplicate wells. Migration assays of CD45RA$^+$ and CD45RO$^+$ T cell chemotaxis was performed and analyzed as described within ECM-like gels, containing collagen type I, FN, and laminin (23).

Western blot analysis of T cell lysates

Human CD45RA$^+$ or CD45RO$^+$ T cells were incubated in starvation medium (RPMI medium without serum) for 24 h (23). Before testing, 5 x 10^6 cells per sample were activated with different concentrations of HSP60 (15 min, 37°C in a 7% CO_2 humidified atmosphere). Freezing the plates at -70°C for 10 min terminated the reaction. The thawed cells were incubated (60 min, 4°C) in lysis buffer containing EDTA (0.5 mM), NaCl (150 mM), NaF (10 nM), Tris pH 7.5 (25 mM), Triton X-100 (1%), PMSF (200 μg/ml), and phosphatase inhibitor cocktail (1%; Sigma), cleared by centrifugation (30 min, 14x10^3 rpm), and the supernatants were analyzed for protein content. Sample buffer was then added, and, after boiling, the samples, containing equal amounts of proteins, were separated on 10% SDS-PAGE gel and transferred to nitrocellulose membranes. The membranes were blocked [TBST buffer containing low-fat milk (5%), Tris pH 7.5 (20 mM), NaCl (135 mM), and Tween 20 (0.1%)] and probed with the following antibodies: anti-phosphorylated Pyk2 (pPyk2) and anti-total Pyk2 (tPyk2): 1.5 and 0.2 μg/ml, respectively. For TLR2 and TLR4 analysis in T cell lysates, 30 μg of protein from each sample were loaded on 10% SDS-PAGE gel and transferred to nitrocellulose membranes. The membranes were blocked and probed with mAbs against TLR2 and TLR4 (500 μg/ml). Immunoreactive protein bands were visualized using horseradish peroxidase-conjugated secondary Ab and the enhanced ECL system.

Actin polymerization.

CD45RA$^+$ T cells (3x10^6 cells/ml) were preincubated with HSP60 (1 h, 37°C in a 7.5% CO_2 humidified atmosphere) and treated with 200 ng/ml of SDF-1α for 15 s at 37°C and then fixed by the addition of a threefold volume of 3.7% PFA for 10 min at 22°C. Next, the cells were

extensively washed, the membranes were permeabilized, and the T cells were stained with FITC-phalloidin (2 μg/ml), washed, and analyzed by FACScan at 525 nm, using Cell Quest Software, as we described previously (23).

Flow cytometry

Purified human T cells (10^6 cells/sample) were stained (30 min, 4°C) with mouse mAb anti-human LFA-1, CXCR4, CCR5, and L-selectin (20 μg/ml each); washed with PBS (containing 0.05% BSA and 0.05% sodium azide); and incubated (30 min, 4°C) with an FITC-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Lab. Inc., West Grove, PA). After being washed, T cell staining was analyzed using a Becton Dickinson FACSort (Mountain View, CA) using the Cell Quest software.

RESULTS

HSP60 induces T cell adhesion to FN

We purified T cells from the peripheral blood of healthy human donors, fractioned the T cells into $CD45RA^+$ and $CD45RO^+$ subpopulations, incubated the T cells with various concentrations of human HSP60 for various times, and assayed adhesion of the T cells to immobilized ECM components: FN, laminin, or type 1 collagen. We found that HSP60 did not affect cell viability but enhanced T cell adhesion specifically to FN; there was little or no effect on T cell adherence to laminin or collagen type 1 (not shown). The adhesion experiments presented here use, therefore, immobilized FN as the ECM substrate.

Figure 1A shows the effects of various concentrations of human HSP60 on T cell adhesion to FN, measured as the percentage of T cells adhering to FN (background adhesion: 10±3%). It can be seen that the nonactivated $CD45RA^+$ population responded to relatively low concentrations of HSP60 (0.1-1.0 ng/ml). Adhesion decreased at concentrations of 10-100 ng/ml and then increased again at concentrations of 1000-5000 ng/ml. The nonactivated $CD45RO^+$ population responded mostly to the higher concentrations (>100 ng/ml) of HSP60.

$CD45RO^+$ T cells include memory T cells that are responsive to activation by IL-2; we therefore tested whether preactivation with IL-2 might influence the response of the $CD45RO^+$ T cells. Figure 1A shows that activation by IL-2 increased the sensitivity to HSP60 of the $CD45RO^+$ T cell population by 1000 fold. Thus the state of a T cell can influence the way it responds to different concentrations of HSP60.

Time-course experiments demonstrated that 1 h of treatment with HSP60 (30 min pretreatment with HSP60 plus 30 min of incubation on FN) induced peak levels of adhesion (Fig. 1B). Therefore, we used this incubation, unless indicated otherwise.

Figure 1C compares the degree of $CD45RA^+$ T cell adhesion induced by 1 ng/ml HSP60 compared with previously known inducers of T cell adhesion in the 1 h assay. It can be seen that HSP60 was as effective as were optimal concentrations of the pro-inflammatory chemokines SDF-1α (100 ng/ml) or RANTES (100 ng/ml) or the cytokine IL-2 (100 IU/ml). PMA (50 ng/ml) was more effective than these physiological protein molecules, and the control

recombinant molecules VEGF or βFGF (both at 100 ng/ml) were no more effective than the background. Thus HSP60 seems to induce T cell adhesion to FN to about the same degree as do other physiological signals for T cell adhesion.

HSP60 mediates adhesion via a TLR2 pathway

HSP60 has been found to activate macrophages and other cells via TLR4 or TLR2 (17). T cells, however, have been reported to express TLR2 but not TLR4 (19). Figure 2A shows that we could detect both TRL4 and TLR2 proteins in unseparated peripheral blood mononuclear cells (PBMC), but purified T cells were much richer in TLR2 than in TLR4.

To test whether TLR2 or TLR4 might be functionally involved in the activation of T cell adhesion by HSP60, we assayed the effect of preincubating $CD45RA^+$ T cells with blocking antibodies to the TLR molecules. Figure 2B shows that T cell adhesion to FN induced by HSP60 was inhibited by an antibody to TLR2 but not by an antibody to TLR4. This suggested that TLR2 and not TLR4 might be important for HSP60-induced adhesion.

We also tested the effect of HSP60 on the activation of adherence to FN of thymocytes taken from experimental animals with mutations in TLR molecules. Mice of the C3H/HeJ strain harbor a point mutation in TLR4, rendering it nonfunctional, whereas the related C3HeB/FeJ strain has an intact TLR4 molecule (24). It has been reported that Chinese hamsters carry a frameshift mutation in TLR2 resulting in a protein without transmembrane and intracellular domains (25). We isolated thymocytes from 6-wk-old Chinese hamsters and tested their ability to adhere to FN in response to various stimuli. Figure 2C shows that these cells did not adhere to FN in response to recombinant HSP60, but we found that they responded normally to PMA and ConA (data not shown). These results are compatible with the conclusion that TLR2, and not TLR4, is important for T cell adhesion to FN induced by HSP60.

Finally, Fig. 2D shows that the anti-TLR2 antibody inhibited specifically HSP60-induced adherence to FN of purified human $CD45RA^+$ T cells but not adherence induced by SDF-1α or IL-2. Thus the anti-TLR2 antibody was specific for HSP60-induced adherence.

HS60-induced adhesion is not due to contaminating LPS

LPS is known to activate cells via both TLR4 and TLR2 (1, 17, 18). It was therefore critical to learn whether contamination of the recombinant HSP60 by LPS might have been responsible for the activation of T cell adhesion in our experiments. Figure 3A shows the amounts of LPS detected in the recombinant HSP60 preparations we used. It can be seen that 1.161 EU/ml of LPS could be detected in GroEL but that LPS was essentially undetectable in the other two preparations: human HSP60 and MT-HSP65 (i.e., <0.1 pg/ml).

We also tested the dose-response curves of CD45RA+ and CD45RO+ human T cells to purified LPS itself. Figure 3B shows the effect on T cell adhesion to FN of various concentrations of LPS. The undulating form of the LPS dose-response curve and the greater sensitivity of the CD45RA+ T cells were similar to what was observed in response to human HSP60. Note, however, that a 1000-fold greater concentration of LPS was needed and the maximal adhesion

response was still less than that induced by human HSP60 (compare Figs. 1A and 3B). Thus, LPS contamination of the HSP60 was not likely to account for the induction of T cell adhesion.

To discriminate between HSP60 and LPS in yet another way, we tested the effects of mAb anti-HSP60 antibody, boiling (that denatures HSP60 but not LPS), or the LPS inhibitor polymyxin B (PMB; ref 17) on both preparations. Figure 3C shows that the effect of HSP60 on T cell adhesion was inhibited by boiling but not by PMB; the opposite was noted with LPS. We also found that mAb anti-human HSP60 specifically inhibited the proadhesive effect of HSP60 but not LPS. Taken together, the results shown in Fig. 3A, B, and C lead to the conclusion that the effect of HSP60 in activating T cell adhesion could not be explained by LPS contaminating the HSP60 preparation.

GroEL but not MT-HSP65 induces T cell adhesion

Figure 4A shows the response to low concentrations of the HSP60 molecule of *E. coli* (GroEL). It can be seen that GroEL also enhanced the adhesion of the $CD45RA^+$ population, but to a lesser degree than the adhesion induced by human HSP60 (compare Figs. 1A and 4A). However, not all species of HSP60 appear able to activate adhesion of $CD45RA^+$ T cells at nanograms per milliliter concentrations; the HSP60 molecule of MT-HSP65 was not active in this assay (Fig. 4B). These findings support the conclusion that mammalian HSP60 can be more effective on T cells than some closely related (1) prokaryotic HSP60 family molecules.

HSP60-induced T cell adhesion depends on β1 integrins

Information about molecular mechanisms involved in the T cell response to HSP60 is shown in Fig. 5. Figure 5A shows that HSP60-induced adhesion was significantly inhibited by monoclonal antibodies to VLA4, VLA5 (the FN-specific α4 and α5 chains of β1 integrins), or CD29 (the common β1 integrin chain); in contrast, control monoclonal antibodies to VLA2 (α2β1), VLA3 (α3β1), or CD44 did not affect HSP60-induced adhesion. It is noteworthy that 1 h treatment of the two T cell subpopulations with HSP60 did not affect the expression levels of FN-specific α4β1 and α5β1 integrins (data not shown). Thus, existing FN-specific β1 integrins on T cells would appear to be activated in HSP60-induced adhesion. This adhesion appears to involve phosphatidylinositol 3-kinase (PI-3 kinase) and protein kinase C (PKC) signaling because it was inhibited (Fig. 5B) by the compounds wortmanin and GF109203X (GF) but not by pertussis toxin (PTX), which inhibits specifically G-protein-coupled signaling (19).

The cytoplasmic tyrosine kinase 2 (Pyk-2) is a member of the focal adhesion kinase (FAK) family of molecules that are phosphorylated upon PKC activation (26–28). Tyrosine phosphorylation of Pyk2 (and related molecules) increases the activity of this kinase and links β1 integrins to multiple signaling pathways regulating adhesion and migration processes in T lymphocytes. Such intracellular processes are also involved in the regulation of mitogen-activated protein (MAP) kinases (such as ERK-2) and Jun-NH_2 kinase pathways (29–31). Figure 5C shows that a short treatment (15 min) of nonactivated $CD45RA^+$ T cells with HSP60 (0.01-100 ng/ml) induced the phosphorylation of Pyk-2. Note that similar to T cell adhesion to FN (see Fig. 1A), Pyk-2 phosphorylation showed a bell-shaped dose-response to rising concentrations of HSP60, reaching peak phosphorylation at 1 ng/ml HSP60. Thus, Pyk-2 phosphorylation accompanies the response of T cells to effective concentrations of HSP60.

HSP60 inhibits chemokine-induced T cell chemotaxis and actin polymerization

The ability of T cells to navigate through the ECM depends on combined signals mediated by proadhesive mediators like cytokines and chemoattractants, associated with ECM glycoproteins. With the use of a three-dimensional, ECM-like gel system designed to follow in real time the migration of individual leukocytes along chemotactic gradients in vitro, we have found that IL-2, RANTES, or SDF-1α gradients can activate human $CD4^+$ T cell chemotaxis along the ECM (23). We found that HSP60 alone, presented into the chemokine zone (drop III) at the beginning of the assay, did not activate $CD45RA^+$ T cell chemotaxis (not shown), although it did induce adhesion (see Fig. 1A). We then tested the effect of HSP60 treatment on T cell chemotaxis. Untreated or HSP60-treated $CD45RA^+$ T cells were embedded in ECM gels, onto which a chemokine depot (SDF-1α or ELC, Fig. 6A and B, respectively) was previously introduced, and the directional migration of the cells was monitored over time as described previously (28). Figure 6A shows that ~30% of untreated $CD45RA^+$ T cells moved toward the SDF-1α source; there was no directional movement in the absence of SDF-1α ($P<0.05$). Treatment of the human T cells with a concentration of 0.1 ng/ml of HSP60, however, inhibited 66% of the T cells migrating toward SDF-1α ($P<0.01$). Thus, a concentration of HSP60 that induces adhesion also inhibits T cell migration toward SDF-1α within an ECM-like gel.

CD45RA+ T cells have also been shown to respond to the chemokine ELC (also designated CCL19), a prototypic lymph node chemokine that attracts migrating dendritic cells and CD45RA+ T cells to T cell areas within the lymph node (30). Figure 6B shows that HSP60, at 0.1 ng/ml, also significantly inhibited (60% inhibition at $t=30$ min; $P<0.05$) the migration of T cells toward ELC. Thus, HSP60 induced inhibition of ECM chemotaxis to both SDF-1α and ELC.

T cell adhesion to and migration through immobilized ECM ligands requires actin polymerization and subsequent cellular polarization (21, 30). To study actin polymerization, $CD45RA^+$ T cells were left intact or treated with HSP60 (1 h, 1 ng/ml) and then activated by SDF-1α (100 ng/ml) for 15 s. The proportion of redistributed F-actin in untreated control T cells was considered to be 100%. Figure 6C shows that HSP60 inhibited actin polymerization triggered by SDF-1α. Moreover, this inhibitory effect of HSP60 was itself inhibited by an anti-TLR2 mAb but significantly less so by the isotype-matched anti-TLR4 monoclonal antibody. Hence, the effect of HSP60 on the rearrangement of the cytoskeleton and its associated signaling molecules (32) also involves TLR2 signaling.

HSP60 inhibits CXCR4 and CCR7 expression

The inhibition of T cell migration to SDF-1α and ELC (Fig. 6) raised the question of the effect of HSP60 on the expression of chemokine receptors: CXCR4, which is involved in T cell chemotaxis toward SDF-1α (CXCL12, and CCR7, which is involved in immune cell chemotaxis toward CCL19 (ELC). Accordingly, we incubated human T cells with various concentrations of HSP60 and assayed the expression of the chemokine receptors CXCR4 and CCR7 at various times. We found that 18 h of incubation sufficed to reduce specifically the expression of these receptors.

Figure 7A and C, shows that 0.1 ng/ml of HSP60 down-regulated the expression of CXCR4 and CCR7 by 40-50% in $CD5RA^+$ T cells. Inhibition of CXCR4 in $CD45RO^+$ T cells required higher concentrations of HSP60 (1 μg/ml; Fig. 7B). Similar HSP60 treatment of $CD45RA^+$ T cells did not affect the expression of LFA-1 or of L-selectin (Fig. 7D). Figure 7E shows that this down-regulation of expression of CXCR4 on $CD5RA^+$ T cells by HSP60 was blocked by anti-TLR2 but not by anti-TLR4 mAb. The inhibitor of PKC signaling, GF109203X, also inhibited the effect of HSP60 on CXCR4 expression (not shown). Thus, the regulatory effect of HSP60 on CXCR4 also requires TLR2 and PKC signaling.

DISCUSSION

The results presented here show that soluble HSP60 can induce activation of β1 integrins in human T cells and enhance human T cell adhesion specifically to the FN component of the ECM. Associated intra-cellular events included activation of signal molecules, PI-3 kinase and PKC, and the subsequent phosphorylation of Pyk-2, a cytoskeletal molecule associated with β1 integrin activation. HSP60 activated a degree of adhesion similar to that activated by other molecules previously known to augment T cell adhesion, such as the chemokines SDF-1α and RANTES and the cytokine IL-2. Incubation of T cells with soluble HSP60 also inhibited SDF-1α-induced cytoskeletal rearrangement (actin polymerization) and SDF-1α and ELC (CCL19)-induced chemotaxis in ECM-like gels. The expression of the relevant chemokine receptors, CXCR4 and CCR7, was also inhibited by HSP60 treatment. It remains to be learned why the effect of HSP60 in inhibiting T cell chemokine receptor (CXCR4 and CCR7) expression required more time than did blocking of chemotaxis and enhancement of adhesion (18 h compared with 1 h). Nevertheless, both types of effects were inhibited by a specific mAb to TLR2 but not by a mAb anti-TLR4. Thus, the effects of HSP60 on T cells would seem to be TLR2 dependent.

Although bacterial HSP60 molecules are quite similar in sequence and structure to mammalian HSP60 molecules (33), the GroEL molecule of *E. coli* was less potent for human T cells than was human HSP60, and MT-HSP65 was ineffective in inducing adhesion. Apparently, the innate T cell receptor can be more sensitive to self-HSP60 than to foreign HSP60. It is also noteworthy that the T cells were much more sensitive to HSP60 than they were to LPS (Fig. 3B). Moreover, the effect of HSP60 was inhibited by a mAb specific for HSP60 and was sensitive to temperature but not to PMB; the opposite was observed with LPS (Fig. 3C). These findings, taken together, argue against the possibility that the effects of HSP60 we observed on T cells might have been induced by LPS contamination.

It is interesting that the sensitivity of the T cell response to HSP60 was influenced by T cell subset and state of activation. $CD45RA^+$ T cells responded to lower concentrations of HSP60 than did $CD45RO^+$ T cells. However, the $CD45RO^+$ T cells gained enhanced sensitivity after activation by IL-2. Indeed, exposure to IL-2 was reported to induce an enhanced expression of TLR2 mRNA in murine T cells (34).

The complex physiology of the innate response to soluble HSP60 is also highlighted by the 1000-fold greater sensitivity of T cells compared with macrophages. The innate receptor TLR4 appears to be required for the macrophage response to HSP60 (35–38). As we show here, murine T cells with nonfunctional, mutated TLR4 could still respond to HSP60, indicating that TLR4 is

not required for this innate T cell response. Indeed, antibody to the innate TLR2 molecule inhibited the T cell responses to soluble HSP60. This implies that the innate T cell response to HSP60 requires TLR2 signaling. The Chinese hamster was reported to harbor a frameshift mutation leading to a nonfunctional TLR2 molecule (25); we found that agents such as PMA can activate the adhesion of Chinese hamster thymocytes to FN (Fig. 2C). However, HSP60 is ineffective. This further implies that an intact, functional TLR2 is required for HSP60 signaling in T cells. It remains to be seen whether TLR2 serves as a primary receptor for soluble HSP60 or whether TLR2 is a necessary participant in the response of other receptors to the HSP60 molecule.

The bi-phasic, low, and high dose-response curve of T cell reactivity to HSP60 (Fig. 1A) raises the possibility that the HSP60 molecule might be recognized by multiple receptors, demonstrating high and low affinities. The high-affinity receptor might be down-regulated or desensitized by high concentrations of the ligand. It is conceivable that the $CD45RA^+$ T cell population expresses both high and low affinity receptors constitutively, while the $CD45RO^+$ T cells bear only a low-affinity receptor until activated by IL-2 stimulation to express the high-affinity receptor. Other explanations for the different sensitivities of the T cell subsets and for the bell-shaped dose-response curve are possible, and characterization of the innate T cell receptors for HSP60 will help clarify the issue.

TLR molecules are thought to constitute "pattern recognition receptors" directed to "pathogen associated molecular patterns" (32, 39). Yet, TLR4 (14) and, as we find here, TLR2 are likely to be involved in signaling by self-HSP60. The present results, which relate to T cell behavior in vitro, call attention to the possibility that the innate T cell response to HSP60 might have functional consequences. HSP60 expression is up-regulated during inflammation, and HSP60 has been detected in soluble form in the blood in the nanogram to microgram per milliliter range of concentrations (5, 8–10). Thus, soluble HSP60 may modulate the innate arm of the immune system and influence the adaptive autoimmune receptors to HSP60 (40).

It is interesting that HSP60 seems to interact with diverse receptor molecules, like TLR2 and TLR4. It has also been shown that HSP70, gp96, and HSP90 interact with human macrophages via the CD91 and TLR4 molecules and, thereby, affect cytokine and chemokine secretion by these cells and their antigen presentation capacities (41–44). It is conceivable that molecules such as CD91, TLR2, and TLR4 are components of common signaling pathways activated by diverse ligands. The different ligands may bind directly to other molecules that trigger CD91 and the TLR molecules further downstream. Indeed, it appears that HSP60 requires CD14, TLR2, and TLR4 for signaling of macrophages, but HSP60 does not bind directly to these molecules (43, 45). It is conceivable that HSP60 and other stress protein molecules activate TLR signaling by first forming complexes with other carrier molecules (46).

HSP70 has been reported to produce anti-inflammatory effects in models of endotoxemia (47), ischemic injury (48), hypoxia (49), and acute respiratory stress syndrome (50). HSP60-induced activation of MHC-I-expressing APC was shown to affect the ability of antigen-specific $CD8^+$ T cells to secrete IFNγ in the absence of antigenic peptides (51). However, although the effect of HSP60 on macrophages is also likely to enhance inflammatory reactions, the present finding that HSP60 can inhibit T cell chemotaxis points to a more subtle influence of HSP60 on inflammation. Based on the present results, low concentrations of HSP60 would be expected to

impede the mobilization to an inflammatory site of "high affinity" cells like the "naïve" $CD45RA^+$ T cell population. However, low concentrations of HSP60 would not inhibit the chemotaxis of the nonactivated "memory" $CD45RO^+$ T cell population. Indeed, a high concentration of HSP60 at the inflammatory site might prevent the exit of the $CD45RO^+$ T cells from the site by enhancing their adhesion to the ECM. Upon contact with T cell regulatory factors such as IL-2, the $CD45RO^+$ T cells would acquire increased sensitivity to HSP60 signals. These possibilities are currently under investigation.

ACKNOWLEDGMENTS

This study was supported by grants from the Center for the Study of Emerging Diseases (Jerusalem, Israel) and the Minerva Foundation (Germany). I. R. Cohen is the incumbent of the Mauerberger Chair of Immunology and the Director of the Robert Koch-Minerva Center for Research in Autoimmune Diseases. O. Lider is the incumbent of the Weizmann League Career Development Chair in Children's Diseases. G. Nussbaum was supported by the Beth Israel Medical Center, (N.Y., USA) and as of July, 2001 by a JDRF post doctoral fellowship. S. Franitza was supported by a grant from the Minerva Foundation (Germany). Anti-HSP60 mAb, clone P5, was kindly provided by Francisco Quintana.

REFERENCES

1. Wallin, R. P. A., Lundqvist, A., More, S. H., von Bonin, A., Kiessling, R., and Ljunggren, H.-G. (2002) Heat shock proteins as activators of the innate immune system. *Trends Immunol.* **23,** 130–135

2. Kol, A., Bourcier, T., Lichtman, A. H., and Libby, P. (1999) Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells, and macrophages. *J. Clin. Invest.* **103,** 571–577

3. Young, D. B., Ivanyi, J., Cox, J. H., and Lamb, J. R. (1987) The 65 kD antigen of mycobacteria – a common bacterial protein? *Immunol. Today* **8,** 215–219

4. Van Eden, W., Thole, J. E. R., van der Zee, R., Noordzij, A., Embden, J. D. A., and Cohen, I. R. (1988) Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. *Nature* **331,** 171–173

5. Elias, D., Markovits, D., Reshef, T., van der Zee, R., and Cohen, I. R. (1990) Induction and therapy of autoimmune diabetes in the nonobese diabetic (NOD/LT) mouse by a 65-kDa heat shock protein. *Proc. Natl. Acad. Sci. USA* **8,** 1576–1580

6. Abulafia-Lapid, R., Elias, D., Raz, I., Keren-Zur, Y., Atlan, H., and Cohen, I. R. (1999) T-cell responses of type I diabetes patients and healthy individuals to human hsp60 and its peptides. *J. Autoimmun.* **12,** 121–129

7. Raz, I., Elias, D., Tamir, M., Metzger, M., and Cohen, I. R. (2001) β Cell function in new-onset type I diabetes and immunomodulation with a heat shock protein peptide (DiaPep277): a randomized, double blind, phase II trial. *Lancet* **258,** 1749–1753

8. Wick, G. (2000) Atherosclerosis: an autoimmune disease due to an immune reaction against heat-shock protein 60. *Herz* **25,** 87–90

9. Direskeneli, H., Eksioglu-Demiralp, S., Yavuz, T., Egrun, T., Shinnick, T., Lehner, T., and Akoglu, T. (2000) T cell responses to 60/65 kDa heat shock protein derived peptides in Turkish patients with Behcet's disease. *J. Rheumatol.* **27,** 708–713

10. Yokota, S. I., Hirada, D., Minota, S., Higashiyama, T., Kurimoto, M., Yanagi, H., and Kubota, T. (2000) Autoantibodies against chaperonin CCT in human sera with rheumatic autoimmune diseases: comparison with antibodies against other Hsp60 family proteins. *Cell Stress Chaperones* **5,** 337–346

11. Hu, W., Hasan, A., Wilson, A., Stanford, M. R., Li-Yang, T., Todryk, S., Whiston, R., Shinnick, T., Mizushima, Y., van der Zee, R., et al. (1998) Experimental mucosal induction of uveitis with the 60-kDa heat shock protein-derived peptide 336-351. *Eur. J. Immunol.* **28,** 2444–2455

12. Mor, F., and Cohen, I. R. (1992) T cells in the lesion of experimental autoimmune encephalomyelitis: enrichment for reactivities to myelin basic protein and to heat shock proteins. *J. Clin. Invest.* **90,** 2447–2455

13. Konen-Waisman, S., Cohen, A., Fridkin, and M., Cohen, I. R. (1999) Self heat-shock protein (hsp60) peptide serves in a conjugate vaccine against a lethal pneumococcal infection. *J. Infect. Dis.* **179**, 403-413

14. Xu, Q., Schett, G., Perschinka, H., Mayr, M., Egger, G., Oberhollenzer, F., Willeit, J., Kiechl, S., and Wick, G. (2000) Serum soluble heat shock protein 60 is elevated in subjects with atherosclerosis in a general population. *Circulation* **102,** 14–20

15. Lap Laplante, A. F., Moulin, V., Auger, A., Landry, J., Li, H., Morrow, G., Tanguay, R. M., and Germain, L. (1998) Expression of heat shock proteins in mouse skin during wound healing. *J. Histochem. Cytochem.* **46,** 1291–1301

16. De Graeff-Meeder, D., Voorhorst, E. M., van Eden, W., Schuurman, H. J., Huber, J., Barkley, D., Maini, R. N., Kuis, W., Rijkers, G. T., and Zegers, B. J. (1990) Antibodies to the mycobacterial 65 kD heat shock protein are reactive with synovial tissues of adjuvant arthritic rats and patients with rheumatoid arthritis and osteoarthritis. *Am. J. Pathol.* **137,** 1013–1021

17. Ohashi, K., Burkart, V., Flohe, S., and Kolb, H. (2000) Heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. *J. Immunol.* **164,** 558–561

18. Kol, A., Sukhova, G. K., Lichtman, A. H., and Libby, P. (1998) Chlamydial heat shock protein 60 localizes in human atheroma and regulates macrophage tumor necrosis factor-alpha and matrix metalloproteinase expression. *Circulation* **98,** 300–307

19. Misukacharoen, T., Matsuguchi, T., Kikutci, T., and Yoshikai, Y. (2001) NF-kB and STAT5 play important roles in the regulation of mouse toll-like receptor 2 gene expression. *J. Immunol.* **166,** 4516–4524

20. Butcher, E. C., and Picker, L. J. (1996) Lymphocyte homing and homeostasis. *Science* **272,** 60–66

21. Vaday, G. G., and Lider, O. (2000) Extracellular matrix moieties, cytokines and enzymes: dynamic effects on immune cell behavior and inflammation. *J. Leukoc. Biol.* **67,** 149–159

22. Ariel, A., Yavin, E. J., Hershkoviz, R., Franitza, S., Avron, A., Cahalon, L., Fridkin, M., and Lider, O. (1998) IL-2 induces T cell adherence to extracellular matrix: Inhibition of adherence and migration by IL-2 peptides generated by leukocyte elastase. *J. Immunol.* **161,** 2465–2472

23. Franitza, S., Kollet, O., Brill, A., Vaday, G. G., Petit, I., Lapidot, T., Alon, R., and Lider, O. (2001) TGFβ enhances SDF-1α-induced chemotaxis and homing of naïve T cells by upregulating CXCR4 expression and downstream cytoskeletal effector molecules. *Eur. J. Immunol.* **32,** 193–202

24. Qureshi, S. T., Lariviere, L., Leveque, G., Clermont, S., Moore, K. J., Gros, P., and Malo, D. (1999) Endotoxin-tolerant mice have mutations in Toll-like receptor 4. *J. Exp. Med.* **189,** 615–625

25. Heine, H., Kirschning, C. J., Lien, E., Monks, B. G., Rothe, M., and Golenbock, D. T. (1999) Cells that carry a null allele for toll-like receptor 2 are capable of responding to endotoxin. *J. Immunol.* **169,** 6971–6975

26. Van Seventer, G. A., Mullen, M. M., and Van Seventer, J. M. (1998) Pyk2 is differentially regulated by integrin- and CD28-mediated co-stimulation in human CD4 T lymphocytes. *Eur. J. Immunol.* **28,** 3967–3877

27. Seig, D. J., Hauk, C. R., Ilic, D., Klingbell, C. K., Schaefer, E., Damsky, C. H., and Schlaepfer, D. D. (2000) FAK integrates growth-factor and integrin signals to promote cell migration. *Nat. Cell Biol.* **2,** 249–256

28. Zrihan-Licht, S., Fu, Y., Settleman, J., Schinkmann, K., Shaw, L., Keydar, I., Avraham, S., and Avraham, H. (2000) RAFTK/Pyk2 tyrosine kinase mediates the association of p190 RhoGAP with RasGAP and is involved in breast cancer cell invasion. *Oncogene* **19,** 1318–1328

29. Franitza, S., Hershkoviz, R., Kam, N., Lichtenstein, N., Vaday, G. G., Alon, R., and Lider, O. (2000) TNFα associated with extracellular matrix fibronectin provides a stop signal for chemotactically migrating T cells. *J. Immunol.* **165,** 2738–2447

30. Cyster, J. G. (1999) Chemokines and cell migration in secondary lymphoid organs. *Science* **286,** 2098–2102

31. Serrador, J. M., Nieto, M., and Sanchez-Madrid, F. (1999) Cytoskeletal rearrangement during migration and activation of T lymphocytes. *Trends Cell Biol.* **9,** 228–232

32. Medzhitov, R., and Janeway, C. (2000) Innate immunity. *N. Engl. J. Med.* **343,** 338–344

33. Jindal, S., Dudani, A. K., Harley, C. B., Singh, B., and Gupta, R. S. (1989) Primary structure of a human mitochondrial protein homologous to bacterial and plant chaperonins and to hsp65 KD mycobacterial antigen. *Mol. Cell. Biol.* **9,** 2279–2283

34. Matsuguchi, T., Takagi, K., Musikacharoen, T., and Yoshikai, Y. (2000) Gene expressions of lipopolysaccharide receptors, toll-like receptors 2 and 4, are differently regulated in mouse T lymphocytes. *Blood* **95,** 1378–1385

35. Chow, J. C., Young, C. D., Golenbock, D. T., Christ, W. J., and Gusovsky, F. (1999) Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J. Biol. Chem.* **274,** 10689–10692

36. Yang, R. B., Mark, M. R., Gray, A., Huang, A., Xie, M. H., Zhang, M., Goddard, A., Wood, W. I., Gurney, A. L., and Godowski, P. J. (1998) Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signaling. *Nature* **395,** 284–288

37. Scher, H. P., Sharrock, C. E., and Panayi, G. S. (1992) High frequency of cord blood lymphocytes against mycobacterial 65-kDa heat-shock protein. *Eur. J. Immunol.* **22,** 1667–1669

38. Ramage, J. M., Young, J. L., Goodall, J. L., and Gaston, J. S. (1999) T cell responses to heat-shock protein 60: differential responses by $CD4^+$ T cell subsets according to their expression of CD45 isotypes. *J. Immunol.* **162,** 704–710

39. Muzio, M., Polentarutti, N., Bosisio, D., Prahladan, M. K. P., and Mantovani, A. (2000) Toll-like receptors: a growing family of immune receptors that are differentially expressed and regulated by different leukocytes. *J. Leukoc. Biol.* **67,** 450–456

40. Cohen, I. R. (2000) *Tending Adam's Garden: Evolving The Cognitive Immune Self,* Academic Press, London, UK

41. Basu, S., Binder, R. J., Ramalingam, T., and Srivastava, P. K. (2001) CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. *Immunity* **14,** 303–313

42. Kol, A., Lichtman, A. H., Finberg, R. W., Libby, P., and Kurt-Jones, E. A. (2000) Heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear cells. *J. Immunol.* **164,** 13–17

43. Habich, C., Bagart, K., Kolb, H., and Burgart, V. (2002) The receptor for heat shock protein 60 on macrophages is saturable, specific, and distinct from receptors for other heat shock proteins. *J. Immunol.* **168,** 569–576

44. Binder, R. J., Han, D. K., and Srivastava, P. K. (2000) CD91: a receptor for heat shock protein gp96. *Nat. Immunol.* **1,** 151–155

45. Heldwein, K. A., Golenbock, T. D., and Fenton, M. J. (2001) Recent advances in the biology of toll-like receptors. *Mod. Asp. Immunobiol.* **1,** 249–252

46. Srivastava, P. (2002) Role of heat-shock proteins in innate and adaptive immunity. *Nat. Rev. Immunol.* **2,** 185–194

47. Lau, S. S., Griffin, T. M., and Mestril, R. (2000) Protection against endotoxemia by HSP70 in rodents cardiomyocytes. *Am. J. Physiol. Heart Circ. Physiol.* **278,** 1439–1445

48. Christians, E. S., Yan, L. J., and Benjamin, I. J. (2002) Heat shock factor 1 and heat shock proteins: Critical partners in protection against acute cell injury. *Crit. Care Med.* **30,** 43–50

49. Delogu, C., Signore, M., Mechelli, A., and Famularo, G. (2002) Heat shock proteins and their role in heart injury. *Curr. Opin. Crit. Care* **8,** 411–416

50. Weiss, Y. G., Maloyan, A., Tazelaar, J., Raj, N., and Deutschman, C. S. (2002) Adenoviral transfer of HSP-70 into pulmonary epithelium ameliorates experimental acute respiratory distress syndrome. *J. Clin. Invest.* **110,** 801–806

51. More, S. H., Breloer, M., and von Bonin, A. (2001) Eukaryotic heat shock proteins as molecular links in innate and adaptive immune responses: HSP60-mediated activation of cytotoxic T cells. *Int. Immunol.* **13,** 1121–1217

Received January 9, 2003; accepted April 11, 2003.

Fig. 1

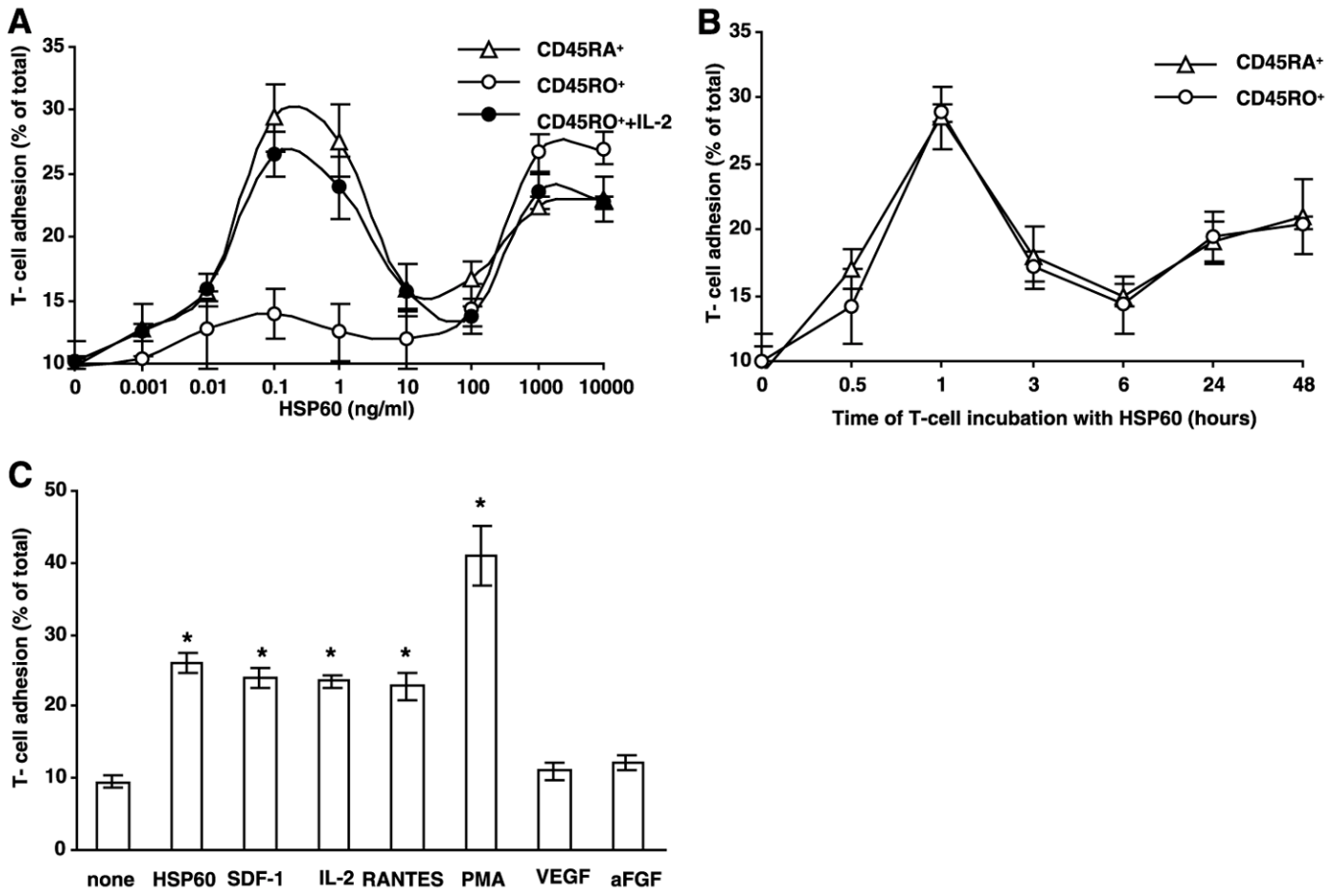

Figure 1. HSP60 induces adhesion to FN of CD45RA$^+$ and CD45RO$^+$ human T cells. A) Purified human CD45RA$^+$ and CD45RO$^+$ T cells were incubated (24 h; tissue culture conditions) with or without IL-2 (10 ng/ml), washed, and labeled with ^{51}[Cr]. Cells were then pretreated (30 min) with different concentrations of HSP60 and seeded onto FN-coated microtiter wells for an additional 30 min. The nonadherent cells were washed away, and the remaining FN-bound cells were lysed. Radioactivity of lysates, representing the amount of FN-adherent cells, was determined. The mean ± SD of 6 independent experiments, using different donor bloods, is shown. **B)** Time-kinetic analysis of HSP60-induced T cell adhesion. Tcells were exposed to 0.1 ng/ml (CD45RA$^+$) or 1 μg/ml (CD45RO$^+$) of HSP60. The optimal increase in adhesion was at 1 h (30 min preincubation + 30 min of adhesion in the plate). Results are percentage of adhered T cells. The mean ± SD of 5 independent experiments is shown. **C)** HSP60 induces T cell adhesion to FN similar in magnitude to other proinflammatory activators. CD45RA$^+$ T cells were treated with 0.1 ng/ml HSP60, 100 ng/ml SDF-1α, 100 IU/ml IL-2, 100 ng/ml RANTES, 50 ng/ml PMA, 100 ng/ml VEGF, or 100 ng/ml FGF for 30 min and tested for adhesion to FN. The mean ± SD of 3 independent experiments is shown.

Fig. 2

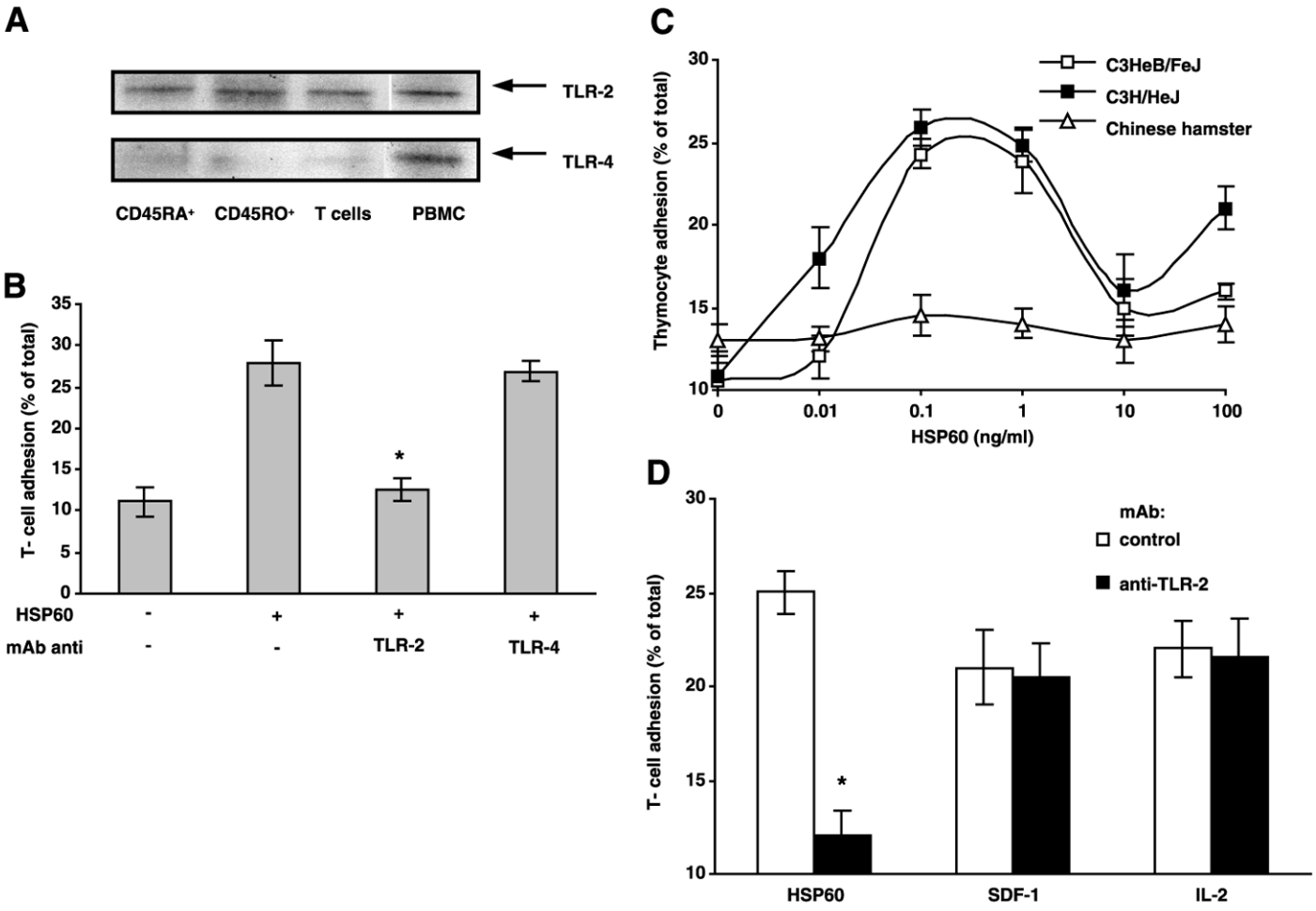

Figure 2. TLR2, but not TLR4, is involved in HSP60-induced T cell adhesion. A) 5 x 10^6 $CD45RA^+$ or $CD45RO^+$ T cells, unseparated T cells, or PBMC were lysed, and the level of TLR2 and TLR4 expression in the cells was determined by Western blotting using specific mAb against TLR2 and TLR4. The SDS-PAGE profile shows a representative experiment of 3 that produced similar results. **B)**. $CD45RA^+$ T cells were pretreated with mAb anti-TLR2 or anti-TLR4 (20 μg/ml). The cells were then treated with HSP60 (0.1 ng/ml), and percent adhesion to FN was determined. The mean ± SD of 4 independent experiments is shown. **C)** Thymocytes of wild-type C3HeB/FeJ, or TLR4-mutated C3H/HeJ mice (>95% $CD45RA^+$ T cells) or thymocytes of Chinese hamsters were radiolabeled, pretreated with HSP60, and seeded onto FN-coated microtiter wells. The mean ± SD of 3 different experiments is shown. **D)** Anti-TLR2 mAb treatment inhibits HSP60-induced adhesion of $CD45RA^+$ T cells but not adhesion induced by SDF-1α or IL-2. The T cells were treated with HSP60 (0.1 ng/ml), SDF-1α (100 ng/ml), or IL-2 (100 IU/ml), and tested for adhesion. Open bars: control mAb. The mean ± SD for 5 different experiments is shown.

Fig. 3

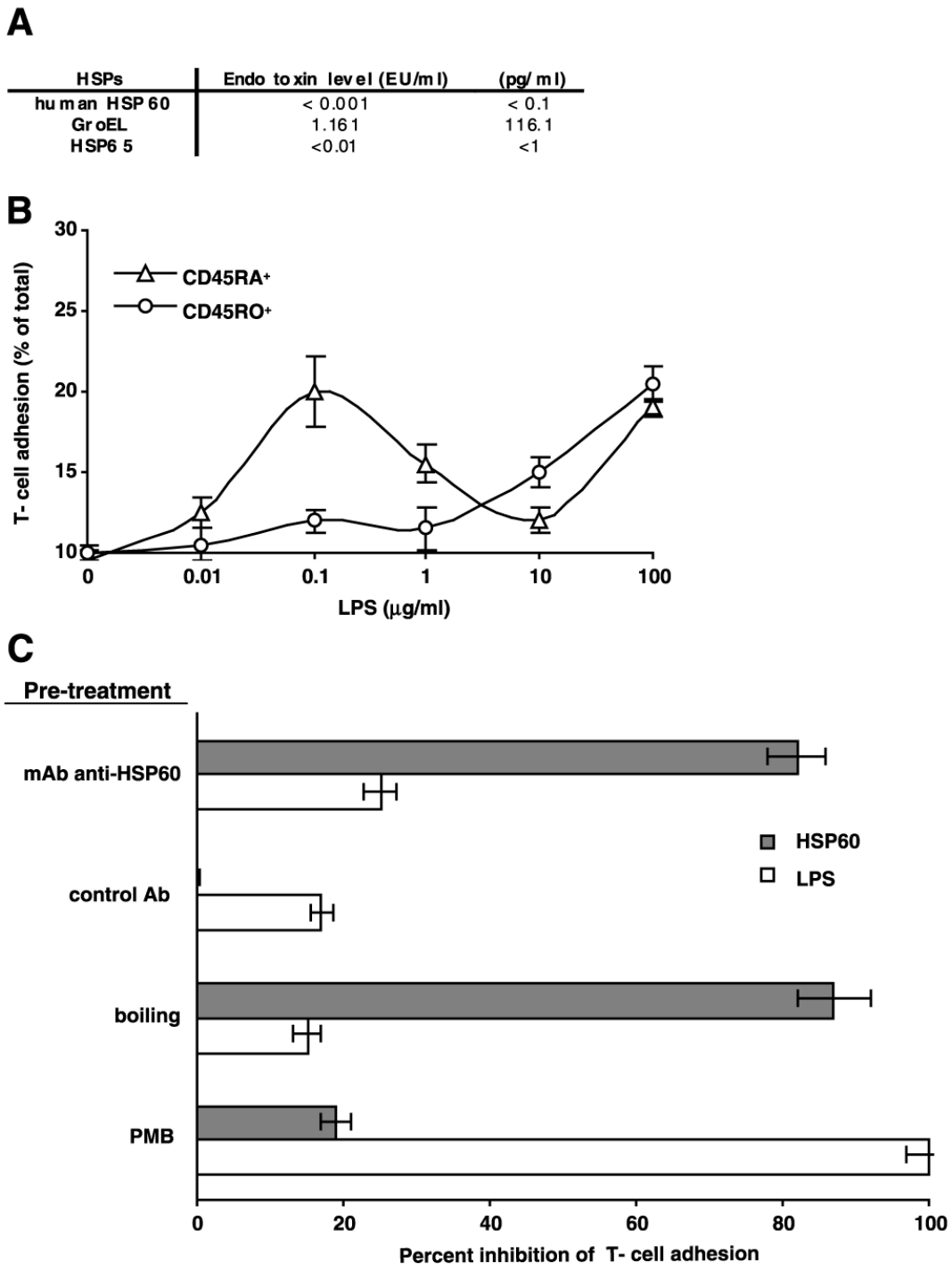

Figure 3. HSP60-induced T cell adhesion to FN is not explained by endotoxin. A) Endotoxin levels of recombinant human HSP60, GroEL, and MT-HSP65 were measured by the kinetic-turbidimetric LAL lysate assay. **B)** Purified human T cells were radiolabeled, pretreated with different concentrations of LPS, and seeded onto FN-coated microtiter wells. The mean ± SD of five experiments is shown. **C)** Purified human T cells were radiolabeled, treated with 0.1 ng/ml of HSP60 or 100 ng/ml of LPS, and seeded onto FN-coated microtiter wells. As indicated, T cells were pretreated with 20 μg/ml of mAb anti-HSP60 (clone P5), or 20 μg/ml of isotype matched mAb (IgM), or 1 μg/ml of polymyxin B (PMB) for 30 min before the addition of HSP60 or LPS. As indicated, HSP60 and LPS were heat treated (100°C, 30 min) before addition to T cells. Results are a percentage of inhibition of T cell adhesion induced by HSP60 or LPS. The mean ± SD of 3 different experiments is shown.

Fig. 4

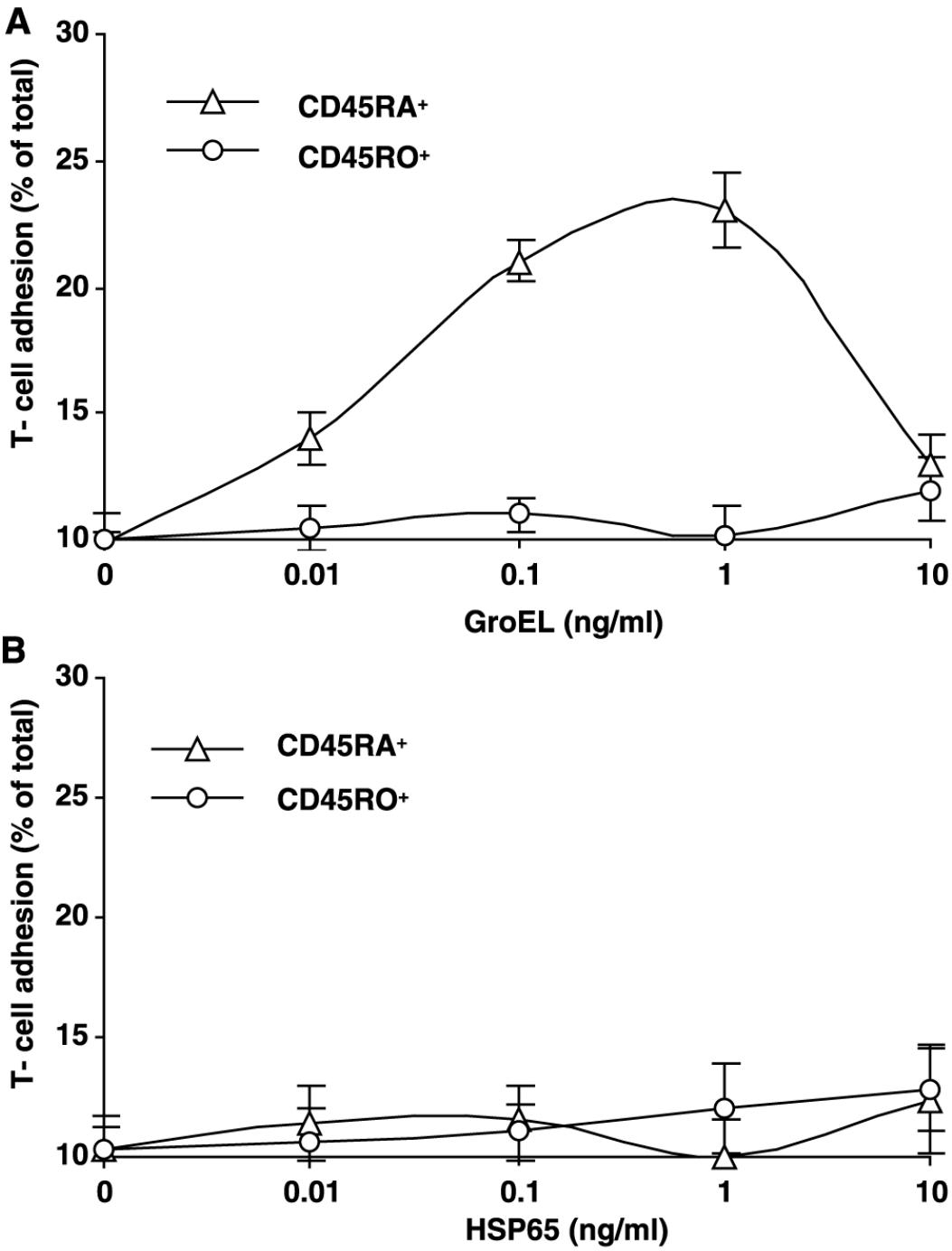

Figure 4. GroEl, but not Mycobacterium tuberculosis (MT-HSP65), induces CD45RA$^+$ and CD45RO$^+$ T cell adhesion to FN. Purified human T cells were radiolabeled, treated with different concentrations of GroEL (**A**) or MT-HSP65 (**B**), and tested for adhesion to FN. The mean ± SD of 5 experiments is shown.

Fig. 5

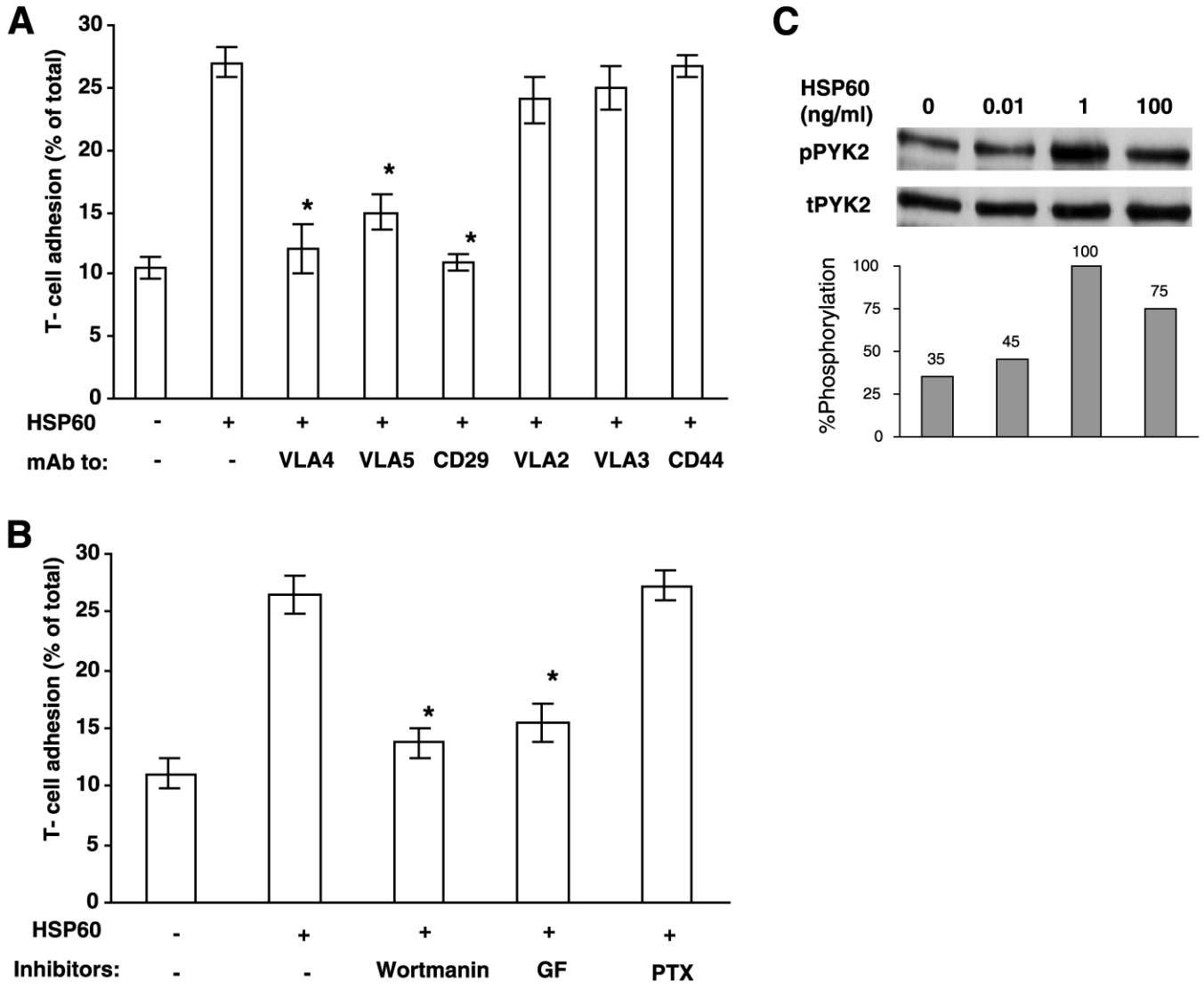

Figure 5. Signaling mechanisms involved in β1-integrin-mediated HSP60-induced CD45RA$^+$ T cell adhesion to FN. CD45RA$^+$ T cells were pretreated (**A**) with the indicated mAb (10-15 μg/ml) or (**B**) intracellular signal transduction inhibitors wortmanin (5 nM), GF109203X (20 nM), or pertussis toxin (2 μg/ml). The cells were then activated with HSP60 (0.1 ng/ml) and tested for binding to FN. The results are percentage of adhered T cells. The mean ± SD of 3 different experiments is shown. **C)** CD45RA$^+$ T cells were exposed to HSP60 (15 min) and lysed. The lysates were run on SDS-PAGE, transferred to nitrocellulose membranes, and immunobloted with mAb anti-phospho-PYK2 (pPYK2) and anti-total PYK2 (tPYK2). The densitometric histograms of the experiment are expressed as (pPYK2/tPYK2) × 100. One representative experiment of 5 is shown.

Fig. 6

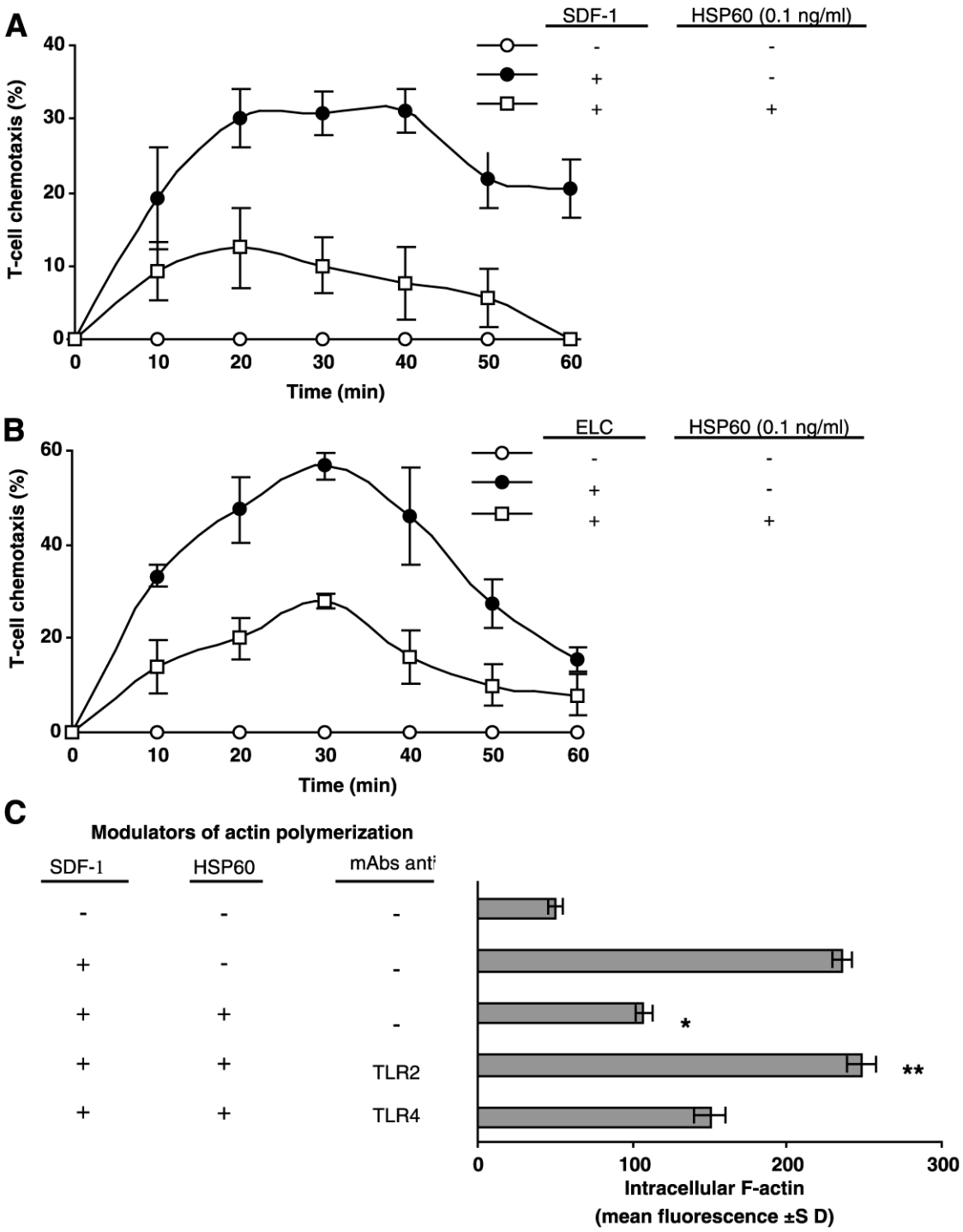

Figure 6. HSP60 modulates CD45RA$^+$ T cell chemotaxis to SDF-1α and ELC and actin polymerization induced by SDF-1α. CD45RA$^+$ T cells were treated (1 h) with HSP60, washed, and applied (250 cells in 10 μl gel of drop I) in migration chambers. The percentage (±SD) of T cell chemotaxis (directionally-migrating T cells) toward (**A**) SDF-1α or (**B**) ELC out of the total number of cells tested was analyzed at 60 min after connecting the 3 drops of the ECM-like gel within the chamber. The data at each time point are the average migration of 100-150 cells. The combined mean ± SD of 5 different experiments is shown. **C)** CD45RA$^+$ T cells were treated (1 h) with HSP60, washed, and exposed for 15 s at 37ºC to SDF-1α (200 ng/ml) in the presence or absence of mAb anti-TLR2 and TLR4 (20 μ/ml; 15 min before treatment with HSP60). The cells were then fixed, and intracellular F-actin was stained with FITC-phalloidin and analyzed (10^6 cells per sample) by FACS. Results are the average of the mean fluorescence ± SD of 3 experiments.

Fig. 7

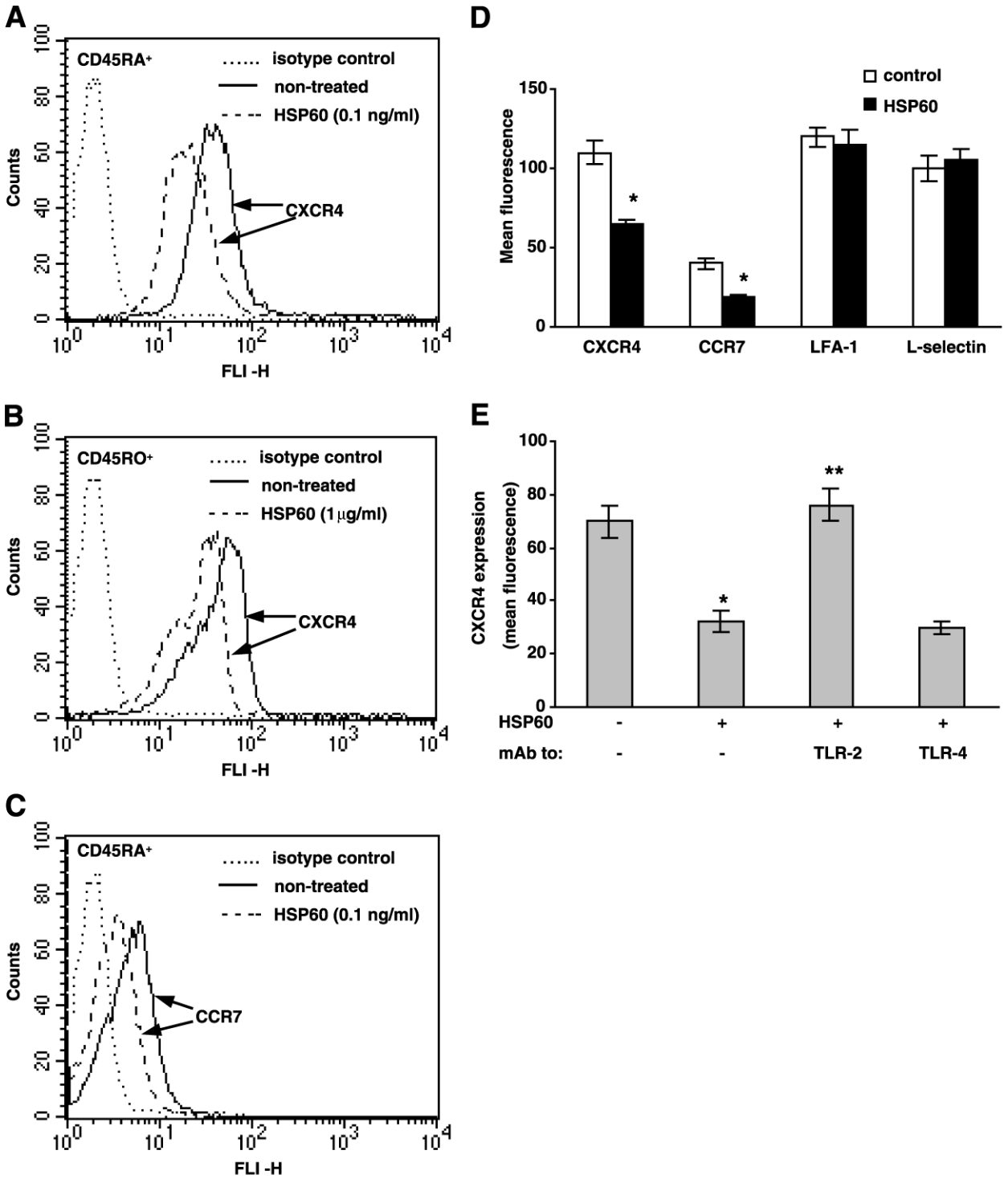

Figure 7. HSP60 down-regulates CXCR4 and CCR7 expression, but not LFA-1 and L-selectin expression, on human T cells in a TLR2-dependent manner. CD45RA$^+$ (**A, C**) and CD45RO$^+$ (**B**) T cells were treated (18 h) with 0.1 ng/ml or 1 μg/ml, respectively, of HSP60, washed, and stained with 20 μg/ml mAb anti-CXCR4 (**A, B**), anti-CCR7 (**C**), anti-LFA-1, or L-selectin (**D**), followed by a secondary FITC-conjugated Ab. Receptor expression was determined by FACScan analysis. One representative experiment of 5 is shown. **E)** CD45RA$^+$ T cells were pretreated (1 h) with mAb anti-TLR2 or anti-TLR4 (20 μg/ml). The cells were then activated with HSP60 (0.1 ng/ml for 18 h), and CXCR4 expression was determined as above. The results are shown as the average of mean fluorescence ± SD of 3 experiments.