Expression of CD137 on Hodgkin and Reed–Sternberg Cells Inhibits T-cell Activation by Eliminating CD137 Ligand Expression

Weng Tong Ho1,4, Wan Lu Pang1,4, Siew Meng Chong2, Antonio Castella6, Suhail Al-Salam6, Teng Ee Tan1,4, Mei Chung Moh1,4, Liang Kai Koh1,4, Shu Uin Gan3, Cheong Kin Cheng1,4, and Herbert Schwarz1,4,5

Abstract

Hodgkin lymphoma is caused by a minority population of malignant Hodgkin and Reed–Sternberg (HRS) cells that recruit an abundance of inflammatory cells. The long-term survival of HRS cells among the vast majority of immune cells indicates that they have developed potent immune escape mechanisms. We report that the TNF receptor family member CD137 (TNFRSF9) is expressed on HRS cells, while normal B cells, from which HRS cells are most often derived, do not express CD137. In 48 of 53 cases of classical Hodgkin lymphoma, CD137 was detected on HRS cells. Ectopically expressed CD137 transferred by trogocytosis from HRS cells to neighboring HRS and antigen-presenting cells, which constitutively express the CD137 ligand (CD137L and TNFSF9), became associated with CD137L and the CD137–CD137L complex was internalized. Disappearance of CD137L from the surface of HRS and antigen-presenting cells led to reduced costimulation of T cells through CD137, reducing IFN-γ release and proliferation. Our results reveal a new regulatory mechanism for CD137L expression that mediates immune escape by HRS cells, and they identify CD137 as a candidate target for immunotherapy of Hodgkin lymphoma. Cancer Res; 73(2): 652–61. ©2012 AACR.

Introduction

Hodgkin lymphoma is characterized by a minority population of malignant Hodgkin and Reed–Sternberg (HRS) cells in a background of inflammatory cells. HRS cells are, in most cases, derived from B cells and secrete cytokines and chemokines that stimulate and recruit the background inflammatory cells (1, 2). A puzzling finding in Hodgkin lymphoma and many or, possibly, all cancers is the presence of tumor-specific immune cells in patients that can kill the tumor cells in vivo. Yet, these immune cells do not eliminate the cancer in vivo.

Similarly, most tumor immunotherapy approaches aim at enhancing the anticancer immune response but have had little success in human patients (3, 4). One common reason for the inability of immune cells to eliminate cancers and the inefficiency of tumor immunotherapy is that malignant cells are immunoedited and selected to avoid elimination by the immune system (5). Among the immune escape mechanisms that cancers, including Hodgkin lymphoma, develop are the secretion of immune inhibitory molecules such as interleukin 10 (IL-10), galectin-1 (6, 7), or prostaglandin E2 (8), the expression of molecules such as PD-1 ligands that induce apoptosis in infiltrating immune cells (9, 10), or the induction of regulatory T cells that inhibit an antitumor immune response (11).

The TNF receptor family member CD137 (4-1BB, TNFRSF9) is a T-cell costimulatory molecule and CD137 agonists potently enhance IL-2 secretion, survival, and the cytolytic activity of T cells, and are being developed for human tumor immunotherapy (12–15). CD137 ligand (CD137L, 4-1BBL, and TNFSF9) is a member of the TNF superfamily, and is expressed on the surface of antigen-presenting cells (APC), and the CD137 receptor/ligand system is used by APCs to costimulate T-cell activity during cognate interactions (16, 17). Bidirectional signaling exists for many members of the TNF and TNF receptor families, which means that both molecules, receptor and ligand, send a signal through their partner molecule but also transmit a signal into the cell they are expressed on (18).

Reverse signaling through CD137L enhances the activity of APC (19, 20). It induces monocyte activation, proliferation, and migration (21–23). In addition, it induces human monocytes to differentiate to dendritic cells and, then, immature dendritic cells to mature dendritic cells (24–26). In human B cells, CD137L signaling enhances proliferation and immunoglobulin secretion, mediating costimulatory signals that are initiated by CD137-expressing helper T cells or follicular dendritic cells (27, 28).

Here, we show that CD137 is ectopically expressed by HRS cells in 48 of 53 cases of classical Hodgkin lymphoma. Ectopically expressed CD137 associates with the constitutively expressed CD137L on HRS cells, and the CD137–CD137L
complex becomes internalized. Ectopically expressed CD137 also gets transferred by trogocytosis to surrounding APC where it also leads to the disappearance of CD137L and reduced costimulation of T cells evidenced by reduced IFN-γ secretion, proliferation, and cytolytic activity. These data identify a novel regulatory mechanism of CD137L expression. Further, we propose a hypothesis that ectopic expression of CD137 contributes to the escape of HRS cells and classical Hodgkin lymphoma from immune surveillance.

Materials and Methods

Tissue samples
Paraffinized tissue sections were obtained from residual or archival patient samples from the Department of Pathology, Faculty of Medicine and Health Sciences, United Arab Emirates University, UAE and the Department of Pathology, YLL SoM, National University of Singapore, Singapore.

Immunohistochemistry
Antigen demasking was done by pressure cooking (WMF, Germany) in pH6 Target Retrieval Solution (DakoCytomation) for 15 minutes at 109°C and cooling for 5 minutes before pressure release. After 5 minutes of 3% hydrogen peroxide block, the tissue was incubated overnight with mouse anti-human CD137 antibody (Lab Vision; clone BBK-2, diluted with TBS containing 1% human serum to a final concentration of 2 μg/mL) at room temperature (RT: 22–25°C).
Detection was carried out using the HRP-labeled Envision secondary reagent for 30 minutes. Color development was carried out by the DAB+ kit according to the manufacturer’s instructions (DakoCytomation). The tissue was counterstained with Gill’s Hematoxylin.

Cells
The HRS cell lines KM-H2, L-428, and L-1236 were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ), and were authenticated by DSMZ using DNA-typing, PCR analysis, and cytogenetic testing. KMS-11 cells were a gift from Dr. Cheng, NUHS, Singapore. Aliquots of cells were frozen after amplification for 3 passages, and fresh cells were regularly thawed and used for the experiments. The cells were cultured in RPMI-1640 (Sigma) supplemented with 10% to 20% FBS.

KM-H2-control and KM-H2-CD137− cells were generated by nucleofection (Amaxa) of KM-H2 cells with an empty SiStrike vector (Promega) or a vector expressing a CD137-specific siRNA, obtained from Ambion of the following sequence: 5'-AAGCAGTTACTACAAGGATCC. Stable clones were selected with blasticidin (100 μg/mL). KM-H2-control and KM-H2-CD137− cells were generated by lentiviral transduction. Full-length cDNA of CD137 or CD137L was cloned into pLenti6 vector (Invitrogen). The cells were transduced with CD137 or CD137L viral supernatant and subsequently selected with blasticidin (100 μg/mL).

Human peripheral blood mononuclear cells (PBMC) were obtained from National University Hospital (NUH, Singapore), and PBMC were isolated using Histopaque-1077 (Sigma) and spun at 400g for 30 minutes. T cells were isolated from PBMC via magnetic activated cell sorting (MACS) using anti-CD3 microbeads (Miltenyi Biotec).

Antibodies
Anti-human CD137 ligand antibody clone 5F4 was purchased from Biolegend, while clone 4B4-436 was obtained from Enzo Life Sciences. Anti-CD137 antibody clone BBK-2 was obtained from Thermo Fisher Scientific. PE-conjugated anti-CD137 antibody (clone 4B4-1) and anti-CD137L (clone 5F4) was obtained from BD Biosciences and Biologen. Isotype control mouse IgG1 and PE and APC conjugated donkey anti-mouse IgG1 secondary antibody were purchased from Sigma and eBioscience, respectively.

Coculture
For coculture, 5 × 10^5 KM-H2 cells were added to 10^6 PBMC or T cells. Then, 0.5 ng/mL of anti-CD3 antibody (clone OKT3) were added to suboptimally activate the PBMC or T cells.

Flow cytometry
Aliquots of cultured cells were stained with respective fluorochrome-conjugated antibodies in PBS containing 0.5% FBS and 0.02% sodium azide (FACS buffer) for 15 minutes at 4°C in the dark. Cells were then washed and resuspended in 500 μL of FACS buffer. Flow cytometry was carried out on a CyAn ADP Analyzer (Beckman Coulter) with Summit data acquisition and analysis software. Nonspecific staining was controlled by isotype-matched antibodies.

ELISA
The concentrations of IFN-γ in cell supernatants were determined by Human IFN Gamma ELISA Ready-SET-Go (eBioscience) according to the manufacturer’s instructions.

Immunoblotting
Cell samples were lysed using RIPA buffer for 10 minutes at 4°C, and denatured with Laemmli Buffer for 10 minutes at 95°C. Cell lysates were then separated by SDS-PAGE and transferred to preactivated PVDF membranes. Membranes were blocked (5% dry milk and 0.05% Tween 20 in PBS) for 1 hour at RT, and probed with anti-CD137L (1:2,000, clone 4B4-436; Adipogen) or anti-GAPDH (1:2,000, clone 6C5; Santa Cruz Biotechnology) overnight at 4°C. Membranes were then probed with HRP-conjugated goat anti-mouse IgG1 (1:5,000, Santa Cruz Biotechnology) for 1 hour at RT, and were visualized using CL-Xposure film (Thermo Scientific) and SuperSignal West Femto Chemiluminescent Substrate or SuperSignal West Pico Chemiluminescent Substrate (Pierce), depending on exposure condition.

Immunoprecipitation
Cell samples were lysed under nonnaturating conditions (1% Triton-X 100, 0.1 mmol/L PMSF in PBS) for 10 minutes on ice. CD137-CD137L complexes were precipitated with 2 μg of anti-CD137 (clone BBK-2) and 50 μL of Protein G Plus Agarose
head (Pierce) per milligram of cell lysate protein overnight at 4°C. The complexes were eluted from agarose head and denatured by boiling in Laemmli Buffer at 99°C for 10 minutes and analyzed by Western blotting.

Confocal microscopy
KM-H2 cells were fixed with 4% PFA for 15 minutes at 37°C, and then permeabilized with 1% Triton-X 100. The cells were then blocked with 1% BSA for 1 hour at RT, followed by anti-CD137 staining (clone BBK-2) overnight at 4°C. Next, the cells were stained with donkey anti-mouse APC and PE-conjugated anti-CD137L (Clone 5F4) for 1 hour at RT, separately. The cells were counterstained with DAPI for 10 minutes at 37°C, and mounted on polylysine slide by Prolong Gold anti-fade reagent (Invitrogen). The slides were visualized with Olympus Fluoview FV1000 TIRF.

Reverse transcription PCR
mRNA was isolated by using RNeasy Mini Kits (Qiagen) and cDNA was generated with the RevertAid First Strand cDNA Synthesis Kit (Fermentas). Amplification was done by CD137L-specific and cyclophilin-specific primers, for a total of 40 cycles (30 s at 95°C, 45 s at 57°C, and 60 s at 72°C). CD137L forward: 5’AGCTTGCCCGAGGACGTCCC. CD137L Reverse: 5’GACACTCGGTGCAAGCAAGCG. Cyclophilin forward: 5’GTCCAGCA- TTGTGCATGGAC. Cyclophilin reverse: 5’GACAAGGGCTC- AGACAGC. The PCR products were analyzed on a 1% agarose gel preloaded with GelGreen (Biotium).

Inhibition of endocytosis
CD137 on KM-H2 cells was neutralized by 5 μg/mL of anti-CD137 antibody (clone BBK-2) and 50 μmol/L of monodansylcadaverine (MDC; Sigma-Aldrich) was added concurrently. Expression of CD137 and CD137L was analyzed 24 hours later.

Trogocytosis
The Reed–Sternberg cell lines L-428 and L-1236 were stained with PKH-26 (Sigma-Aldrich) according to the manufacturer’s protocol. In brief, 10⁶ cells were resuspended in 200 μL of diluent C, and PKH-26 was added at a final concentration of 2 μmol/L. The reaction was terminated after 3 minutes by adding 200 μL of FBS. The cells were washed thoroughly with culture media 3 times. PKH-26-labeled L-428 and L-1236 cells were then cocultured with CFSE-labeled KMS-11 cells at a ratio of 1:1. Transfer of PKH-26 and CD137 onto KMS-11 cells was measured by using flow cytometry 24 hours later.

Statistical analysis
Statistical significance was determined by a 2-tailed unpaired Student t test.

Results
CD137 is ectopically expressed on HRS cells in classical Hodgkin lymphoma
In an immunohistochemical screen, we detected CD137 expression on HRS cells in classical Hodgkin lymphoma.

CD137 was very strongly expressed by HRS cells on the cell surface as well as in the cytoplasm and the Golgi apparatus (Fig. 1A–C). HRS cells are, in most cases, derived from B cells on which CD137 expression is not or hardly detectable, indicating that CD137 is ectopically expressed by HRS cells. CD137-expressing HRS cells were found in 48 out of 53 cases of Hodgkin lymphoma (Table 1), which suggest that ectopic CD137 expression is not a mere epiphenomenon but is selected for.

Table 1. Classification of classical Hodgkin lymphoma cases and the presence of CD137+ HRS cells

<table>
<thead>
<tr>
<th>Classification</th>
<th>Cases</th>
<th>CD137+ HRS cells</th>
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<tbody>
<tr>
<td>Nodular sclerosis</td>
<td>37</td>
<td>33</td>
</tr>
<tr>
<td>Mixed cellular</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Lymphocyte-rich</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Not subclassified</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
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<td>48</td>
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Expression of CD137 inhibits CD137L expression on HRS cells

Primary HRS cells are extremely rare and difficult to obtain. Therefore, we employed the HRS cell line KM-H2 for functional studies. Similar to the primary HRS cells, KM-H2 cells express CD137 constitutively. In order to investigate the effects of CD137 in HRS cells, we established a stable line (KM-H2-CD137\(^{−}\)/C0) in which CD137 expression was silenced by siRNA (Fig. 1D). KM-H2 cells transfected with an empty vector (KM-H2-control) that underwent the same selection and cloning procedure as KM-H2-CD137\(^{−}\)/C0 cells served as negative controls (Fig. 1D).

Characterizing the 2 cell lines we noticed higher expression of CD137L on KM-H2-CD137\(^{−}\)/C0 than on KM-H2-control cells, suggesting that the presence of CD137 may reduce expression of CD137L (Fig. 1D). In addition, when CD137 on KM-H2 cells was neutralized by anti-CD137 antibodies, CD137L expression increased confirming the inhibitory influence of CD137 expression on expression of CD137L (Fig. 1E).

Inhibition of CD137L expression by CD137 reduces the T-cell stimulatory capacity of HRS cells

CD137 is a potent T-cell costimulatory molecule, and is expressed at higher levels on CD8\(^{+}\) than on CD4\(^{+}\) T cells. Interaction of CD137L on APC with CD137 on activated T cells induces a Th1 response (12–15). Enforced expression of CD137L on tumor cells facilitates their elimination by enhancing the antitumor immune response, and establishes a protective immune memory (29, 30). On the basis of these data, one would expect that the expression of CD137L on HRS cells negatively affects tumor growth by enhancing an antitumor immune response. Therefore, a mechanism that leads to the disappearance of CD137L expression might support tumor growth.

This hypothesis was tested by coculturing KM-H2-control cells (CD137\(^{+}\) and CD137L\(^{−}\)) or KM-H2-CD137\(^{−}\) cells (CD137\(^{−}\) and CD137L\(^{\text{high}}\)) with preactivated PBMC or T cells and measuring the release of IFN-\(\gamma\), a cytokine indicative of a Th1 immune response. The 2 KM-H2 cell lines did not produce...
IFN-γ nor did the suboptimally preactivated PBMC or T cells. However, in a coculture, the 2 KM-H2 cell lines induced the release of IFN-γ from preactivated T cells and PBMC. Significantly more IFN-γ release was induced by the CD137Lhigh KM-H2-CD137L/controls cells than by the CD137Llow KM-H2-control cells (Fig. 2A). When the 2 cell lines were pretreated with neutralizing anti-CD137 antibody that enhanced CD137L expression on the KM-H2-control cells, the KM-H2-control cells induced comparable amounts of IFN-γ secretion as did the KM-H2-CD137L cells. As expected, the anti-CD137 antibody had no effect on the KM-H2-CD137L cells, which do not express CD137 (Fig. 2A).

That reduced IFN-γ secretion was indeed due to reduced CD137L expression in CD137L-expressing cells was verified by the addition of anti-CD137L antibodies to cocultures of KM-H2 cells with T cells or PBMC. When KM-H2-CD137L cells that had gained in CD137L expression after silencing of CD137 were cocultured with preactivated T cells or PBMC in the presence of anti-CD137L antibodies, induction of IFN-γ secretion was reduced or prevented. This finding was confirmed with 2 different clones of anti-CD137L antibodies (Fig. 2B).

**CD137 associates with CD137L and leads to internalization of the CD137-CD137L complex**

Investigating the mechanism responsible for the disappearance of CD137L when CD137 was present, we tested the influence of CD137 on de novo CD137L expression. Western blot analysis showed increased CD137L levels when CD137 was
neutralized by the antagonistic anti-CD137 antibody (Fig. 3A), thereby confirming the results obtained by flow cytometry (Fig. 1E). However, expression of CD137L mRNA was not changed (Fig. 3B), implying that CD137 does not influence the de novo synthesis of CD137L, but rather affects its turnover.

An increased turnover of proteins is often due to their increased internalization. Because a major internalization pathway is that of endocytosis, we treated KM-H2 cells with monodansyl cadaverine (MDC), an endocytosis inhibitor, and tested CD137 and CD137L levels by flow cytometry. MDC led to an increased expression of CD137L (Fig. 3C, top), suggesting that the reduction of CD137L is, indeed, due to an increased turnover, and that 1 step in this increased turnover is an increased internalization of CD137L. However, not only levels of CD137L but also levels of CD137 increased after inhibiting endocytosis, indicating that CD137 gets internalized as well (Fig. 3C). Treatment of the KM-H2 cells with the neutralizing anti-CD137 antibody increased levels of CD137 and CD137L much further than endocytosis inhibition, and simultaneous inhibition of endocytosis had no additional effect (Fig. 3C).

We corroborated internalization of CD137 and CD137L by confocal microscopy. Both proteins were found on the cell surface as well as in the cytoplasm of KM-H2 cells. In addition, CD137 and CD137L colocalized, showing that the 2 proteins associate and become internalized as a complex (Fig. 3D).

Association of CD137 and CD137L was confirmed by immunoprecipitation of CD137 from KM-H-2 cells and subsequent SDS-PAGE and detection by anti-CD137L antibody (Fig. 3E). Interestingly, pretreatment of KM-H2 cells by the antagonistic anti-CD137 antibody had also increased CD137L levels, and had reduced endocytosis of CD137 and CD137L, we concluded that this antibody prevents the binding of CD137 to CD137L and, thereby, the internalization of CD137L. Similarly, when CD137 was knocked down by siRNA, there was no complex formation and internalization of CD137L, and the HRS cells retained their T-cell costimulatory activity. Therefore, by ectopically expressing CD137, HRS cells acquired a mechanism that eliminates CD137L expression. Because CD137 associates with CD137L, which then leads to the internalization of CD137L and, thereby, to a reduced costimulation of infiltrating T cells, HRS cells are able to evade immune surveillance by ectopically expressing CD137.
Ectopically expressed CD137 is transferred from HRS cells to surrounding CD137L-expressing APC and inhibits their T-cell–stimulatory activity

Because, in Hodgkin lymphoma, HRS cells are a small minority of malignant cells surrounded by a large majority of stromal cells, including monocytes, macrophages, and B cells, which constitutively express CD137L, the disappearance of CD137L from HRS cells alone may not necessarily affect T-cell costimulation significantly. Therefore, we tested whether CD137L expression on surrounding APC is also affected by ectopic expression of CD137 on HRS cells. We identified HRS cells lines (L-428 and L-1236) that do not endogenously express CD137, and established stable CD137-expressing lines (Fig. 4A).

We cocultured control L-1236 and L-1236-CD137 cells with PBMC for 24 hours, and stained the cells for CD137 and the B cell marker CD20 or the monocyte marker CD14. The L-1236 and L-1236-CD137 cells do not express CD20 or CD14. Only B cells and monocytes that had been cocultured with L-1236-CD137 cells were positive for CD137 (Fig. 4B). The transfer of CD137 could already be observed after 4 hours, although at a lower level (not shown).

Ectopic CD137 expression on HRS cells and subsequent reduction of CD137L expression on HRS cells and surrounding APC reduced the activities of cocultured immune cells. Secretion of IFN-γ by preactivated PBMC was reduced in the presence of CD137-expressing L-1236 or L-428 cells (Fig. 4C). Also the proliferation of T cells among the PBMC was significantly lowered by CD137-expressing L-1236 cells (Fig. 4D). Similarly, the mean fluorescence intensities of CFSE-labeled PBMC were 442 and 550 when cocultured with control L-428 and L-428-CD137 cells, respectively. These data show that HRS cells can transfer CD137 to surrounding APC, which leads to a reduction of their costimulatory activity (Fig. 5).

CD137 is transferred to CD137L-expressing APC by trogocytosis

We investigated whether HRS cells transfer selectively CD137 or whether CD137 is transferred as part of an entire membrane patch, a process described as trogocytosis (31, 32), and whether the transfer of CD137 requires the recipient cell to express CD137L.

We used the multiple myeloma cell line KMS-11 that does not express CD137 and expresses only low levels of CD137L, and generated a stable cell line constitutively expressing CD137L at high levels (Fig. 6A). CD137Llow (KMS-11-control) and CD137Lhigh (KMS-11-CD137L) KMS-11 cells were labeled with CFSE, and were cocultured with control L-428 and CD137-expressing L-428 cells that had been labelled with PKH-26. CD137 could be detected on both lines of KMS-11 cells, but significantly higher amounts of CD137 were transferred to the KMS-11-CD137L cells, showing that the transfer of CD137 is increased by CD137L (Fig. 6A). No CD137 was detectable on KMS-11 cells after coculture with control L-428 cells. The transfer of CD137 was paralleled by the transfer of PKH-26, which was highest when the KMS-11 cells (the recipients) expressed high levels of CD137L and when the L-428 cells (the donors) expressed CD137 (Fig. 6A). The same pattern of CD137 and PKH-26 transfer was observed when the L-428 cells were replaced by L-1236 cells, suggesting that the transfer of CD137 is a general feature of HRS cells, and that it occurs by trogocytosis (Fig. 6B).

Discussion

CD137 is expressed by T cells on activation while CD137L is expressed constitutively by APC. APC, in particular dendritic cells, use the CD137 receptor/ligand system to costimulate activated T cells, including anticancer immune responses (29, 30, 33). This constitutive expression of CD137L and its potent T-cell-stimulatory activity pose a problem for APC that undergo malignant transformation, as the antitumor activity of infiltrating T cells can be enhanced by CD137L. Therefore, malignant APCs that get rid of CD137L expression gain a selection advantage. Due to the inhibitory effect of CD137
expression on CD137L expression. One possibility to eliminate the immune stimulatory effects of CD137L is the overexpression of CD137.

This hypothesis is supported for classical Hodgkin lymphoma by (1) a high incidence of CD137 expression on HRS cells, (2) the reduced levels of CD137L on CD137-expressing HRS cells (3), the increase of CD137L levels on inhibition or neutralization of CD137, and (4) the correlation of the T-cell-stimulatory capacity of HRS cells with their CD137L expression. In addition, ectopic CD137 expression allows HRS cells to transfer CD137 to neighboring APC and, thereby, to inhibit CD137L expression and to reduce the overall T-cell activation in the tumor microenvironment.

The expression of CD137 on various leukemic cell lines has been described previously (34). More recently, Anderson and colleagues in 2012 conducted a very comprehensive study on the expression of CD137 on malignant cells in patients with hematologic malignancies (35). Interestingly, these authors find CD137 expression on HRS cells in 86% of classical Hodgkin lymphoma cases, which tallies exactly with our findings.

The same need or advantage to eliminate CD137L expression would arise for myeloid cells on transformation. However, no myeloid leukemias are known to express CD137. It is possible and likely that other mechanisms exist to down-regulate CD137L expression or to neutralize its immune-stimulatory effects.

One such potential mechanisms is the secretion of soluble CD137 (sCD137) which is antagonistic to cell surface expressed CD137 (16, 20, 36) and, indeed, elevated levels of sCD137 have been identified in the sera of patients suffering from hematologic malignancies, especially in chronic lymphocytic leukemia (37).
antitumor immune response and, accordingly, will be selected for or against.

We hypothesize that the transfer of CD137 to CD137L-expressing cells is not restricted to tumors, rather that it is a physiologic mechanism that is used by Hodgkin lymphoma as it provides growth and/or selection advantages. The transfer of CD137 to CD137L-expressing cells is likely a physiologic negative feedback mechanism involved in the limitation of immune responses. As such, it should be present and detectable during ongoing immune responses as they occur in autoimmune diseases.

Trogocytosis is best studied between dendritic cells and T cells. The intensity of trogocytosis, that is, the extent of membrane transfer correlates with the TCR-MHC affinity and, accordingly, trogocytosis has been used to measure the T-cell specificity and diversity (42, 43). We find that transfer of membrane material, that is, trogocytosis is enhanced by the presence of CD137 on the donor and CD137L on the recipient cell, which is most likely due to a stronger association of the 2 cells mediated by CD137–CD137L interaction. This is the first study that implicates the CD137 receptor/ligand system in the recently described process of trogocytosis, and trogocytosis in the pathogenesis of classical Hodgkin lymphoma. Unfortunately, an animal model for Hodgkin lymphoma is not available, which would allow testing the neutralization of CD137 on HRS cells, and its effects on the antitumor immune response in vivo. However, a bispecific antibody targeting CD137 and CD30, another marker for HRS cells, could be developed and tested in vitro and in xenograft models.

This study (1) identifies CD137 being ectopically expressed on HRS cells in classical Hodgkin lymphoma (2), shows a new regulatory mechanism of CD137L expression and T-cell costimulation (3), suggests a novel immune escape mechanism for Hodgkin lymphoma, and (4) identifies the inhibition or neutralization of CD137 on HRS cells as a potential immunotherapy approach for classical Hodgkin lymphoma.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: W.T. Ho, W.L. Pang, H. Schwarz
Development of methodology: W.T. Ho, W.L. Pang, L.K. Koh
 Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): W.T. Ho, W.L. Pang, S.M. Chong, A. Castella, S. Al-Salam, L.K. Koh
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): W.T. Ho, W.L. Pang, H. Schwarz, L.K. Koh
Writing, review, and/or revision of the manuscript: W.T. Ho, L.K. Koh, H. Schwarz
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.E. Tan, L.K. Koh, C.K. Cheng, M.C. Moh, S.U. Gan, M.C. Moh, L.K. Koh, C.K. Cheng
Study supervision: H. Schwarz

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