論文の内容の要旨

論文題目 ： Antimetastatic activity of intestinal bacteria and their components

腸管内細菌とその構成成分による転移の抑制について

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はじめに

一般に、広範囲の放射線照射は生体の免疫能などを抑制し、腫瘍の増殖と転移を促進するとされている。しかし、マウス放射線腹部照射後に線維肉腫細胞（ＮＦＳａ）を静注すると、肺転移数は減少する。この現象は無菌動物や、抗生物質投与動物ではみられず、これには腹部照射後の腸内細菌 Enterobacter cloacae の増殖、粘膜通過、体内への侵入が関与していると報告されていた。論文提出者は大腸菌を腸内フローラとして持つ動物（通常のマウス）では、大腸菌がこの役割をすること、同様の転移抑制効果はこれらの生菌または死菌をあらかじめ静注しておいても見られること、及び細胞成分のうち内毒素ＬＰＳが主としてこの効果を示していることを明らかにした。またその機序のついて研究を進め、ＬＰＳの転移抑制効果にはＮＫ細胞やマクロファージは直接には関与せず、細胞の毛細血管を通しての定着が関与することを示した。

実験方法

動物と腫瘍：マウスは、８－１２週齢の Ｃ３Ｈ／ＨｅＭｓＮｒｓ と Ｃ３Ｈ／ＨｅＪ male を使用した。腫瘍細胞としては、通常Ｃ３Ｈ／Ｋａｍ female nice に自然発生した低免疫原性線維肉腫（ＮＦＳａ）第１７世代の single cell suspensionを作り使用した。

肺転移数は、腫瘍静注１１日後に肺を摘出し、肺表面の転移結節数を計測した。

菌：E.cloacae，E.coli を L-broth で培養しPBSで洗浄して使用した。また E.cloacae から乾燥菌体、ＬＰＳ、ＯＭ、ＩＭを分離した。

実験結果

マウス腹部照射後の大腸菌の変動と転移抑制：　腸管内における E.coli の生菌数を腹部照射（１２Ｇｙ，Ｔ線）後経時的に調べると、照射３－６日後には菌数が１００－１００倍に増加、その後減少し照射２週間後に元のレベルに戻ることがわかった。また、腹部照射後、各臓器における細菌の増殖について調べると、腹部照射前にはまったく細菌が検出されなかったが、照射後３、５、６日目に腸管膜リンパ節より E.coli の生菌が分離された。このような状態下ではＮＦＳａの肺での転移結節は抑制されていた。

生菌死菌及び菌体成分による肺転移抑制能：　E.coli と E.cloacae 生菌静注３日後にＮＦＳａを静注した場合、菌数がマウスあたり10^4で有意の肺転移抑制能を認め10^7ではほぼ完全に肺転移が抑制されることを見いだした。菌数当りの転移抑制能は、E.cloacae と E.coli の間に有意の差は認められなかったが、投与量の増加と共に抑制は強くなった。このような転移抑制効果は死菌を用いた場合にも見られたが、生菌と死菌の肺転移抑制能を比較すると、生菌では約１０倍の転移抑制能が認められた。

次に、菌体成分を分画し転移抑制能を比較したところ、ＯＭに強い抑制能がみられ投与量の増加と共に肺転移数は減少した。その効果は、乾燥死菌投与とほぼ同等で５０μgの静注により大部分の転移が抑制された。ＯＭの主成分であるリポ多糖（ＬＰＳ）を膜から分離し、その活性を調べたところ、ＬＰＳの静注では、ＯＭの重量に対し約１００－１０００分の一の投与で同等の効果がみられＬＰＳが主たる活性物質であると考えられた。しかしＬＰＳに非感受性のＣ３Ｈ／ＨｅＪ miceを用いて E.cloacae のＯＭ，ＩＭの転移抑制能を調べると、ＬＰＳ投与では転移抑制はみられなかったが、ＯＭ，ＩＭには転移抑制効果があり、ＬＰＳ以外にも転移抑制を引き起こす物質があるものと考えられる。

ＬＰＳによる転移抑制機構；ＬＰＳの作用時間と直接作用：　ＬＰＳと腫瘍の静注間隔を変えその経時的変化をみると、腫瘍静注前にＬＰＳを投与した場合、前日投与、３日前投与で強い転移抑制がみられ間隔が開くにつれ転移数は増加した。ＬＰＳ投与２時間後の腫瘍静注では転移抑制は弱く、腫瘍静注２日後のＬＰＳ投与では転移抑制作用はみられなかった。以上の結果より、一度生着した転移細胞にはＬＰＳは作用しないと考えられる。

ＬＰＳの腫瘍細胞への直接作用を調べるため、ＮＦＳａをＬＰＳ加培養液（１－５μg/

ml）で0－72時間培養後、よく洗浄しマウスに静注した。培養時間、培養液中のＬＰＳ濃度を変化させても転移抑制は起こらず、ＬＰＳは腫瘍に直接作用しないことがわかった。

ＬＰＳによる転移抑制機構；ＮＫ細胞、マクロファージの関与：　ＬＰＳで活性化することが知られているマクロファージやＮＫ細胞の関与を調べるため、まずマウスの脾細胞を in vitro でＬＰＳと共に１時間培養後洗浄、別のマウスに移入、転移抑制が起こるかどうかを調べた。腫瘍細胞投与１日前にこの脾細胞を投与するとよく転移を抑制した。しかし、この作用は放射線照射で死亡した脾細胞でもほぼ同様にみられ、またＬＰＳに非感受性のＣ３Ｈ／ＨｅＪ mice への脾細胞移入ではみられなかったので、細胞と結合したＬＰＳによる作用と考えられた。

次に、ＬＰＳ静注後の血液中及び脾細胞中のアシアロＧＭ１陽性細胞の変動をみると、血液中の細胞数はＬＰＳ静注翌日には約２倍となり、５日目に約８倍と最高値になることがわかった。しかし、これらの変動パターンはＬＰＳ投与後の転移抑制効果の時間経過（ＬＰＳ投与１－３日後にピーク）と平衡するものではなかった。

一方、マウスに抗アシアロＧＭ１抗体やシリカを投与しＮＫ細胞やマクロファージの活性を抑制すると、ＮＦＳａ細胞静注後の肺転移数は著名に増加しこれらの細胞が転移形成に関与していることは明かであった。しかしＬＰＳによる肺転移抑制率は抗アシアロＧＭ１抗体やシリカの大量投与では大きく変動せず、ＬＰＳによる肺転移抑制にはこれらの細胞は直接には大きな役割を果たしていないと考えられる。また、マウスの全身照射でＬＰＳによる肺転移抑制が大きく抑えられることから、放射線照射で破壊される何らかの細胞が関与しているものと考えられる。

ＬＰＳによる転移抑制機構；静注した腫瘍細胞の肺への定着と流出：　ＮＦＳａ細胞を ^{125}Ｉでラベルし、これをあらかじめ抗アシアロＧＭ１抗体（１／５０、０．５ｍｌ）、ＬＰＳ（１μｇ）または両者を投与したマウス及び対象マウスに静注、経時的に肺を取り出しγ－カウンターで測定した。対照群と比べＬＰＳのみの投与を受けたマウスでは、腫瘍静注４時間以降に肺の腫瘍細胞数が顕著に低下した。抗アシアロＧＭ１抗体のみを投与されたマウスでは腫瘍静注６時間後でも対照マウスと腫瘍細胞数に差はみられず、２４時間後でも若干の上昇にとどまった。両者を投与した動物でもＬＰＳ単独投与の場合と著明な差は認めず、同抗体の効果はほとんど見られなかった。

ＬＰＳによる肺転移抑制効果の腫瘍特異性：　高免疫原性線維肉腫（ＦＳＡ）、扁平細胞癌（ＮＲＳ１，ＳＳＣⅦ）、乳癌（ＭＭｃａ）、耳下腺癌（ＮＲＰＧ）を使用し、ＬＰ

Sの効果を調べた。その結果、FSA、SSCⅦでは、NFSaと同様、強い転移抑制がみられたが、NRS1、MMcaではその作用は一部のみにとどまり、NRPGの転移数はまったく変わりがなかった。このことはLPSの事前投与は肉腫のみならず癌でもかなり効果があるが、これらが抑制されるかどうかは細胞の種類によることを示している。

考察

この論文ではまず腸内のフローラに E.cloacae ではなく E.coli を持つマウスの場合、腹部照射後同菌が腸管内で増殖生体内に移行することを示し、腹部照射後の転移抑制の現象が E.cloacae に特異的なものでないことを明らかにした。そしてあらかじめマウスに生菌、死菌を静注することにより、同様の転移抑制を行うことができることを示した。さらに菌体成分のLPSがその抑制効果の大部分を担当していることを明らかにした。

致死量の全身照射を受けた動物では大腸菌や他のグラム陰性菌が腸管内で増殖すると共に生体内に侵入することが知られており、このようなマウスでは、無菌マウスと比べ照射後の死期が早まることが報告されている。これに対して放射線を腹部のみに照射した場合には菌の生体内への移行は起こるが、致死には至らずこれに伴いある種の腫瘍細胞では転移が抑制されるものと考えられる。

血管内腫瘍細胞の転移形成には、NK細胞、マクロファージなどによる生体防御機構や、血流による物理的障害を逃れ、微小血管壁への着床、血管壁の通過、転移臓器実質内での発育という複雑な過程を経る必要がある。LPSはその作用が多彩でいくつかの過程で転移に関与していると推定される。抗アシアロGM1抗体やシリカの投与によりNK細胞やマクロファージの活性を抑えると、NFSa静注後の肺転移数が著名に増加することからNK細胞やマクロファージがNFSa静注後の肺転移形成に関与していることはまちがいない。しかし今回の実験で、LPSの転移抑制率は抗アシアロGM1抗体やシリカの投与では大きく変化せず、また肺での腫瘍細胞の定着性はLPS投与により著名に低下するが抗アシアロGM1抗体ではほとんど影響を受けなかったため、LPSの転移抑制効果に対するこれらの細胞の関与は否定的であった。また、LPS投与2時間後の腫瘍静注では強い転移抑制が起こらないことからLPS静注後早期に起こる血液中の顆粒球や血小板の減少、補体の活性化などの関与も直接的にはないものと考えられる。しかし、これらの変化が二次的に血管内皮細胞や基底膜細胞に変化を与え、これにより細胞の肺への定着が妨げられている可能性は否定できない。放射線全身照射によりLPSによる肺転移抑制能が低下することは放射線に感受性のあるこれらの細胞の関与を示唆しているものと考えられる。

Antimetastatic activity of intestinal bacteria and their components

腸管内細菌とその構成成分による転移の抑制について

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(2)

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腸管内細菌とその構成成分による転移の抑制について

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目次

要　旨

マウス放射線腹部照射後に線維肉腫細胞（ＮＦＳａ）を静注すると、肺転移数は減少する。特に、Ｔ線１２Ｇｙで、腹部照射後７日目に腫瘍細胞を静注すると、最も著明な転移抑制がみられ、これには腹部照射後の腸内細菌Enterobacter cloacaeの増殖、粘膜通過、体内への侵入が関与していると報告されている。論文提出者はまず、大腸菌を腸内フローラとして持つ動物では大腸菌がこの役割をしこの現象がE.cloacae に特異的でないこと、同様の転移抑制効果はこれらの生菌または死菌をあらかじめ静注しておいても見られること、及び細胞成分のうち内毒素ＬＰＳが主としてこの効果を持つことを示した。この効果は他の腺維肉腫（ＦＳＡ）や扁平細胞癌（ＳＳＣⅦ）でもほぼ同様に認められた。

次いでＬＰＳの抗転移活性の機構について検討した。まず、ＬＰＳ加培養液中でＮＦＳａを培養したがその転移能は不変で、ＬＰＳは腫瘍細胞に直接作用しないことがわかった。次に、抗アシアロＧＭ１抗体やシリカをＬＰＳを投与されたマウスと投与されないマウスに静注しＮＫ細胞やマクロファージの活性を抑制すると、ＮＦＳａ細胞の肺転移数は著明に増加するがＬＰＳによる肺転移抑制率は殆ど影響を受けないこと、ＬＰＳ投与後マウスの末血中のアシアロＧＭ１陽性細胞と転移抑制能の変動は平衡しないことを示した。また、ＬＰＳ静注により腫瘍細胞の毛細血管を通しての定着性が低下し抗アシアロＧＭ１抗体は定着性に殆ど影響しなかった。これらの結果よりＬＰＳによる転移抑制にはＬＰＳにより活性化されるＮＫ細胞やマクロファージは殆ど関与しないこと、ＬＰＳは肺での腫瘍細胞の定着性を低下させるがこの定着性の低下にはＮＫ細胞は殆ど関与していないことがわかった。転移抑制において、腫瘍細胞の定着性の変化とＮＫ細胞やマクロファージによる腫瘍細胞の傷害はまったく別のメカニズムであると考えられているが、この実験のように一種のＢＲＭであるＬＰＳと、転移に関してはＮＫ細胞やマクロファージに感受性があると考えられる腫瘍細胞を用い、これらがまったく別のメカニズムによることを明確に示したのは本論文が初めてである。他方、全身照射（６Ｇｙ）を受けたマウスではＬＰＳによる転移抑制能が低下し、放射線に感受性のある細胞の関与が示唆された。

I. Inhibition of metastasis of weakly immunogenic fibrosarcoma and other tumor cells by intestinal bacteria and their components

ABSTRACT

It has been reported previously that abdominal irradiation caused inhibition of lung metastases and that transmigration and growth of Enterobacter cloacae in mesenteric lymph nodes coincided with this phenomenon, abdominal irradiation-induced inhibition of lung metastases (AIRIM). In this section of the thesis, I show that AIRIM was caused not only by E.cloacae, but also by another intestinal bacteria, E.coli. Furthermore, I found that colony formation in the lung of certain tumor cells which were injected intravenously after intravenous injection of E.coli or E.cloacae, was markedly inhibited. The inhibition was caused by killed bacteria as well as living bacteria. Further analysis disclosed that the inhibition activity was predominantly located in lipopolysaccharide (LPS), which is an important constituent of the outer membrane of these bacteria, although some other components seem to be involved also in the metastatic inhibition by the bacteria. The antimetastatic activity of LPS was found to depend on the tumor cell type; colony formation of fibrosarcoma was extensively inhibited by LPS but that of mammary adenocarcinoma was only partially inhibited. These results suggest that under certain conditions intestinal bacteria play some role in the natural prevention of tumor metastasis.

INTRODUCTION

Exposure of animals to wide-field irradiation before tumor challenge usually causes enhanced tumor growth and metastasis (1). However, Ando et al. found that abdominal irradiation of mice prior to an intravenous challenge of syngeneic fibrosarcoma cells(NFSa) reduced metastases. This phenomenon was designated as abdominal irradiation-induced inhibition of lung metastases(AIRIM) (2,3,4). Neither germ-free mice nor antibiotic-treated mice exerted AIRIM. After abdominal irradiation, transmigration and growth of intestinal E.cloacae in mesenteric lymph nodes was noted and found to be essential for the induction of AIRIM (4).

In this thesis I show that AIRIM was also caused by Escherichia coli, if the bacterium was present in the intestinal flora in the experimental animals, and that intravenous injection of either bacterium reduced lung colony formation of injected tumor cells. I also show that a bacterial component played a significal role in this inhibition.

MATERIALS AND METHODS

Animals. The mice used were 8-to-12-week-old males of strains, C3H/HeJ and C3H/He. They were kept in specific pathogen-free(SPF) facilities or conventional ones. Bacteria isolated from the intestine of the SPF mice were Bacteroides, Bifidobacterium, Clostridium, Enterobacter, Lactobacillus, Staphylococcus, Streptococcus, fusiform bacteria and the flora were free of E.coli. In contrast, the intestinal flora of the conventional mice contained E.coli instead of Enterobacter.

Tumors. A weakly immunogenic fibrosarcoma (NFSa), which arose spontaneously in a C3Hf/Kam female mouse, was kept in liquid nitrogen, and the 17th generation of its cells was used for the present experiments. Strongly immunogenic fibrosarcoma (FSA), squamous cell carcinoma (NRS1 and SSCVII), mammary adenocarcinoma (MMca), and parotid adenocarcinoma (NRPG) that had been stored in liquid nitrogen were also employed.

Single-cell suspensions of tumor cells were prepared as described previously (4). Briefly, tumors were removed, minced with scissors, and then mixed with 20 ml of Dulbecco's solution containing 0.2% trypsin (Difco 1:250), 0.02% pancreatin Grade VI (P-1750; Sigma Chemical Co., St.Louis, MO) and 5 mg deoxyribonuclease I Grade II (Boehringer, West Germany). The beaker was gently stirred at 35°C for 5 min. The supernatant was discarded and fresh solution was added for another 20-min enzyme treatment. The second supernatant was filtered through stainless steel mesh (#200) and centrifuged at 154 x g for 8 min. The pellets were resuspended at the appropriate cell concentration in Hanks' balanced salt solution (HBSS) supplemented with 10 % fetal calf serum (FCS). The viability of cell suspensions always exceeded 95 % (4).

Radiation. For abdominal irradiation, a ^{137}Cs sealed-type γ -cell with a dose rate of 0.75 Gy/min, and a 200 KVp X-ray machine

(HVL 1.2 mm Cu, FSD 81.3cm) with a dose rate of 0.45 Gy/min were employed. Mice were anesthetized by an intrapenitoneal injection of sodium pentobarbital (50 mg/kg). The head, chest, and legs of each mouse were shielded by 5mm of lead from the X-ray beam and by a 50-mm slab from the ^{137}Cs γ-ray beam. This resulted in a dose to the thorax of less than 8 % of the dose specified to the abdomen. To sterilize bacteria, a ^{60}Co γ-ray unit with a dose rate of 60.3 Gy/min was used.

Bacterial Examination. At various times after abdominal irradiation, the bacterial number and species in the cecum were examined. The cecal contents were homogenized and diluted 10-fold with phosphate buffered saline(PBS). Colonies of E.coli and E.cloacae were counted after both aerobic and anaerobic incubation (5).

Dried Bacteria and Lipopolysaccharide(LPS). E.cloacae was grown in L-broth at 37°C for 24hr., harvested by centrifugation (5000rpm, 20 min), and washed 2 times with pyrogen-free saline. The cells were washed with ethanol, acetate, and ether, in this order. They were dried and preserved as dried bacteria at room temperature. The weight of dried bacteria was 28 μg per 10^8 bacteria.

LPS of E.cloacae was extracted from dried bacteria or their outer membrane with phenol-water at 70°C and purified by centrifugation (6). The LPS preparation contained less than 0.1 % protein as determined by the Lowry procedure, and there was no detectable nucleic acid (absorbance at 260nm). The yield of LPS was 0.5 μg from 10^8 bacteria.

Outer Membrane (OM) and Inner Membrane (IM) of E.cloacae. Freshly harvested bacteria were destroyed with a French press and suspended in 10mM HEPES buffer (pH 7.4). The yielding suspension was layered on a discontinuous sucrose density gradient (1.5ml of 2.02M, 5.5ml of 1.44M and 4ml of 0.77M sucrose in the buffer) and centrifuged as described previously (7), and the fractions of OM and IM were collected. After washing 3 times with water, the

fractions were frozen and dried. The yields of OM and IM from 10^8 bacteria were 1.0μg and 0.37μg, respectively. The supernatant fraction in the top layer was also tested for the antimetastatic activity.

Lung Colony Assays. To produce artificial pulmonary metastases, tumor cells were injected intravenously into mice in an injection volume of 0.5ml of HBSS supplemented with 10% FCS. After tumor inoculation, mice were killed at 9 day for NRS1, 11 day for FSA and NFSa, 14 day for SSCVII, 17 day for NRPG, and 25 day for MMCa. The lungs were removed and fixed in Bouin's solution. The number of tumor nodules on the surface of the lungs was counted macroscopically. The significance of the differences between groups (5 mice were used for each group) was determined using the Wilcoxon test; P values less than 0.05 were considered significant.

RESULTS

Numbers of E.cloacae and E.coli Counts in the Intestine Before and After Irradiation. The number of E.cloacae and E.coli in the cecum was determined before and after abdominal irradiation. As shown in Fig. 1, the numbers of each bacterium increased by a factor of 100-1000 at day 3 after irradiation, and they returned to their original levels at day 14. Simultaneously with the increased bacteria in intestinal contents, the same bacteria were found in mesenteric lymph nodes (data not shown), and AIRIM concomitantly took place. I therefore assumed that these bacteria played some role in AIRIM.

Effects of Exogenous Administration of E.cloacae and E.coli on Experimental Metastases. When living E.coli or E.cloacae was injected intravenously 3 days before NFSa challenge, lung metastases were found to be inhibited (Table 1). The inhibition depended on the number of bacteria injected, such that few colonies were found in the lungs of mice that received 10^7 bacteria. A similar inhibition was also found when killed bacteria were employed instead of living bacteria (Table 2), although the activity was 10 times lower than that with living bacteria. These bacteria were injected intravenously 7 days before tumor challenge.

Since killed bacteria were also effective, bacterial components were fractionated and tested for antimetastatic activity. The most prominent activity was found in the outer membrane fraction (OM), and the inner membrane fraction (IM) showed intermediate activity. As shown in Fig. 2, the antimetastatic activities of OM and IM were dose dependent. When lipopolysaccharide (LPS), a constituent of OM, was extracted from E.cloacae and tested for antimetastatic activity, it was found that LPS was much more effective than OM

based on their weights. To learn if LPS was the only effective component, I employed C3H/HeJ, LPS low-responder mice, to compare the antimetastatic effect of LPS with C3H/He mice. In C3H/HeJ mice LPS failed to affect lung colony formation of NFSa, but both OM and IM fractions clearly inhibited metastases in C3H/HeJ mice (Table 3). The results indicated that some other components besides LPS were also involved in the antimetastatic activity of the bacterium.

As shown in Fig. 3, both dried bacteria and LPS, which were administered 1 day before injection of the tumor cells, exhibited the strongest antimetastatic activity, and the longer before the administration was, the less the antimetastatic effect was observed. In this experiment, the antimetastatic activities of 1 μ g LPS and 10^7 dried bacteria were tested during a period ranging from 13 days prior to and 4 days after tumor inoculation. Dried bacteria or LPS administered 2 hours before the injection of tumor cells at day 0 was still effective, but no reduction of metastatic colonies was observed after 2 days.

Metastatic Ability of Various Tumor Cells after Administration of LPS. The effect of LPS on the inhibition of metastases of various types of tumor cells was investigated. Tumor cells tested here included two fibrosarcomas, two squamous cell carcinomas, one mammary adenocarcinoma, and one parotid gland tumor; all were C3H/He-mouse origin. As shown in Table 4, lung colony formation of NFSa, FSA, and SSCVII tumor cells were strongly inhibited, while those of NRS1, MMca, and NRPG cells were marginally inhibited. NFSa and FSA were different in immunogenicity, but they were equally well inhibited, suggesting that the inhibition was not related to the surface difference of these cells.

DISCUSSION

In this section I showed that inhibition of lung metastases was caused by abdominal irradiation, accompanied by the growth of E.coli, as well as E.cloacae, in the intestine and mesenteric lymph nodes. The antimetastatic effect by the irradiation was replace by previous administration of living or killed bacteria. It is believed that transmigration of bacteria to lymph nodes or other sites of the body is caused by irradiation through various mechanisms, including radiation injury to the intestinal mucosa, changes in the intestinal microflora, and depression of host defense mechanisms (8-10).

After more than 1000 roentgens of whole-body irradiation, a majority of the epithelial cells of the intestinal mucosa in mice perished within 3 to 4 days. The survival time of mice receiving a gastrointestinal death-dose of irradiation was proportional to the life span of intestinal mucosal cells(11) and it was shorter for conventional mice than for germ-free mice(12,13) or antibiotic-treated mice(14). After lethal whole body irradiation, the number of bacteria found in feces was larger for sterilized mice than for survived mice. I showed in this thesis that a sublethal irradiation to the abdomen induced the multiplication and transmigration of E.coli (Fig. 1), and the number of E.coli in the intestine returned to the pretreatment level by 14 days, similar to mice that survived after a midlethal dose of whole-body irradiation (15). As a cause of AIRIM, qualitative and quantitative alterations in the microbial population in the gut may be as significant as radiation injury to the intestinal mucosa. After local irradiation to the gut, bacteria translocated to the mesenteric lymph nodes, yet the host defense mechanisms remained intact enough to prevent bacteremia and animal death.

Metastatic tumor cells in the circulation must evade a number

of host defenses, including NK cells, activated macrophages, and lymphocytes. The tumor cells must survive the physical trauma of blood flow and be arrested in the venous or capillary bed of the target organ. Once the tumor cell has been arrested, it must pass through the vascular well to enter the organ parenchyma and grow in this organ (16-18).In the present study, I showed that the injection of living and killed bacteria into mice also inhibited lung metastases, and that LPS could replace bacteria. LPS exhibits a variety of actions, such as the activation of macrophages, NK cells and complements, the induction of TNF, interleukin I and interferon, platelet aggregation, vascular endothelial damage, and augmentation of the adhesion of granulocytes and lymphocytes (19-27). These pleotropic activities of LPS are considered to cause the inhibition of lung metastases. Many investigators have shown that metastases are well inhibited by the activation of NK cells or macrophages (28-30). In the following section II, I studied further whether macrophage and NK cell functions are related to the inhibition of metastases in the present system.

Finally, since OM and IM inhibited metastases in LPS low-responder mice, some other components besides LPS also seem to possess antimetastatic activity. Bacterial components such as peptidoglycan (31) and deoxyribonucreotide (32) have been shown to possess antitumor activity. In particular, muramyl dipeptide, a part of peptidoglycan, which resides in OM, is known to exhibit various activity similar to LPS.

Table 1 Antimetastatic activities of living E.coli and E.cloacae

Mice received living E.coli or E.cloacae 3 days before injection of $1x10^5$ NFSa cells. Mice were sacrificed 11 days after tumor challenge.

Treatment	Number of mice	Mean number of lung colonies(range)	
Saline	5	122.6(105-164)	
10^4 E.coli	5	80.6(40-130)	NS[a]
10^7 E.coli	5	1.6 (1-2)	*[b]
10^8 E.coli	5	0	*
10^4 E.cloacae	5	50.4(42-79)	*
10^7 E.cloacae	5	0	*

a Not significant

b $P<0.01$

Table 2 Effect of prior injection of living or killed E.cloacae on lung colony formation of NFSa cells

Mice received indicated numbers of E.cloacae 7 days prior to injection of $1x10^5$ NFSa cells.

	Mean number of lung colonies(range) after injection of E.cloacae at a dose of:			
E.cloacae	-	10^7	10^8	10^9
-	91.6(49-153)	-	-	-
γ-ray-killed	-	72.6(28-98)[a]	13.6(6-24)[b]	19.6(6-34)[b]
Formalin-killed	-	25.8(3-36)[b]	8.4(4-14)[b]	2.6(0-7)[b]
Dried	-	72.8(52-85)[a]	26.0(16-37)[b]	1.2(0-4)[b]
Living	-	29.8(10-47)[b]	1.0(0-2)[b]	0[b]

[a] Not significant

[b] $P<0.01$

Table 3 Antimetastatic activities of various bacterial components injected to LPS-lowresponder C3H/HeJ and LPS-responder C3H/He mice

50 μg of IM, OM and LPS was injected intravenously 3 days before transplantation of NFSa cells.

Experiment	Injection	Mean number of lung colonies(range) in LPS-lowresponder C3H/HeJ	Mean number of lung colonies(range) in LPS-responder C3H/He
I [a]	Saline	222.0 (183-258)	117.0 (84-143)
	50 μg LPS	212.5 (149-252)	8.5 (1-15)[c]
	50 μg IM	ND	0.8 (0-1)[c]
	50 μg OM	ND	1.2 (0-2)[c]
II [b]	Saline	132.4 (92-158)	ND
	50 μg IM	63.7 (31-123)[d]	ND
	50 μg OM	48.3 (23-79)[e]	ND

[a] $1x10^5$ or [b] $5x10^4$ NFSa cells were injected.

ND: Not done.

[c] $P<0.01$

[d] $P<0.025$

[e] $P<0.005$

Table 4 Effect of LPS on lung colony formation of various tumors

C3H/He mice received 0.2 or 1.0 μg LPS/0.5ml saline 3 days before transplantation of tumor cells.

Tumor		Number of cells injected	Mean number of lung colonies in mice which had received -	0.2 μgLPS	1.0 μgLPS
Fibrosarcoma	(NFSa)	$1x10^5$	139.2(63-191)	7.0(1-12)[a]	0.6(0-3)[a]
Fibrosarcoma	(FSA)	$1x10^6$	52.6(17-84)	3.2(1-5)[a]	1.6(0-4)[a]
Squamous cell carcinoma	(SSCVII)	$2.5x10^5$	79.3(69-100)	3.8(0-9)[a]	4.0(2-6)[a]
Squamous cell carcinoma	(NRS1)	$1x10^5$	123.6(104-134)	60.6(39-71)[a]	70.8(53-83)[a]
Breast adenocarcinoma	(MMca)	$2.5x10^6$	43.8(11-70)	31.2(17-55)[b]	17.6(5-42)[b]
Parotid gland adenocarcanoma	(NRPG)	$1x10^5$	169.6(132-210)	172.0(144-205)[b]	141.2(104-170)[b]

[a] $P<0.01$

[b] Not significant

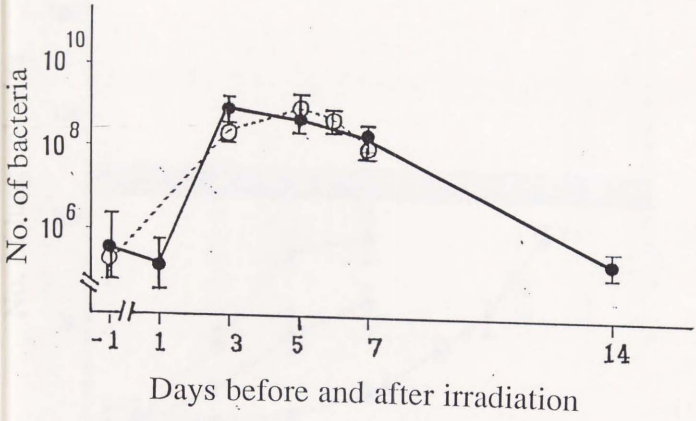

Fig. 1. Numbers of E.coli and E.cloacae in cecal contents before and after abdominal irradiation. At various times before and after abdominal irradiation with 12 Gy of γ-ray, five mice were killed and the number of E.cloacae(●－●) or E.coli(○…○) in cecal contents of SPF and conventional mice were counted by the colony-forming efficiencies. E.cloacae was found only in SPF mice and E.coli in conventional mice. Symbols and bars; mean±SEM.

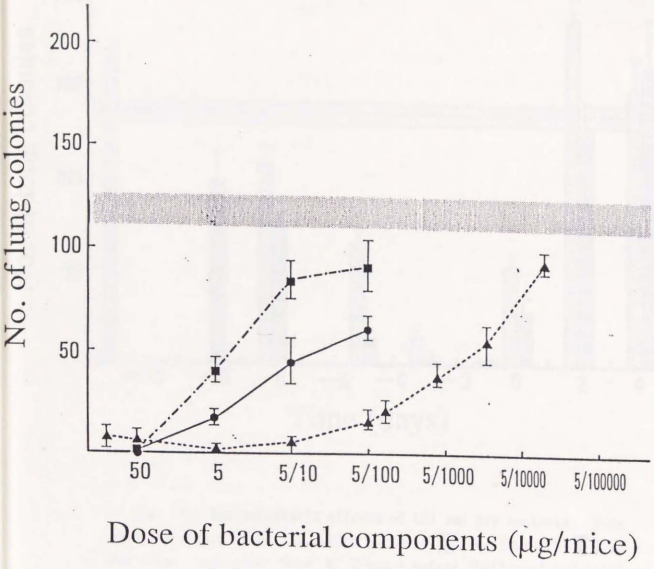

Fig. 2. Effects of IM, OM and LPS on lung colony formation. Various doses of IM(■—■), OM(●—●) and LPS(▲···▲) were injected intravenously 3 days before NFSa cell challenge($1x10^5$). A group of 5 mice was used for a each experiment and plotted as symbols with bars. The stippled band indicate control±SEM. Lung colonies of mice which received the soluble fraction(5μg/mouse) were within the range of the control value.

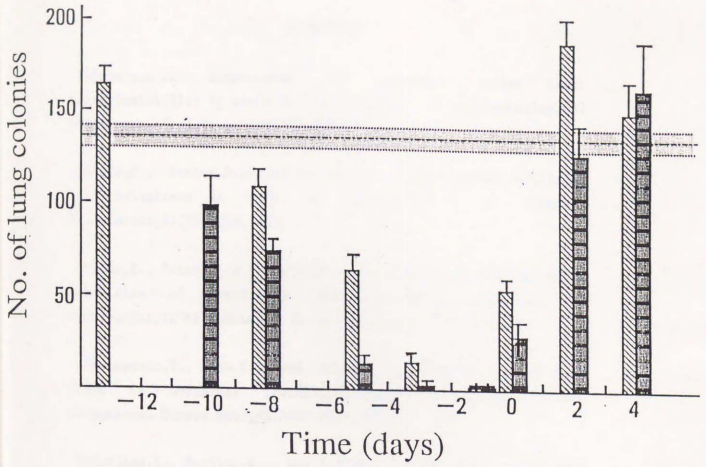

Fig. 3. Antimetastatic effects of LPS and dry bacteria. Mice received either $1x10^7$(2.8μg)dry bacteria(▨) or 1μg LPS(▩) at the time indicated (day 0: 2 hours before challenge) and 1x105 NFSa cells were challenged at day 0. Number of colonies in lung was counted after 11 days. A group of 5 mice was used each experiments. Stippled band indicates control±SEM.

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II. On the mechanisms of the antimetastatic activity of intravenously-injected lipopolysaccharide

ABSTRACT

In the preceding section I showed that bacterial LPS exerted prominent antimetastatic activities. I studied further on the mechanism of the antimetastatic activities of LPS. In vitro treatment of fibrosarcoma (NFSa) cells with LPS did not change their metastatic ability in syngeneic mice, regardless of the incubation time and LPS concentration in the medium. The results indicated no direct effect of LPS on the tumor cells.

Although treatment of mice with anti-asialo GM1 antibody or silica before injection of tumor cells significantly increased lung colony formation whether the mice had or had not received LPS, the rate of colony reduction by LPS was scarcely affected by the treatments. Furthermore, a maximum increase of asialo GM1 positive cells was observed 5 days after injection of LPS, whereas peak inhibition of metastasis was observed 1-2 days after injection. These results suggest that LPS-sensitive cells, such as natural killer(NK) cells or splenogenic macrophages, were not directly involved in the present antimetastatic activity of LPS. The experimental results on the pulmonary retention of radiolabeled NFSa cells showed that LPS affected retention of the tumor cells in the lung, and that this was not suppressed by anti-asialo GM1 antibody. Thus, the antimetastatic activities of LPS were primary due to inhibition of the initial arrest of tumor cells in the lung. The inhibition was partially suppressed by whole-body irradiation.

INTRODUCTION

Prior to metastatic establishment, tumor cells in the circulation must disseminate to distant organs and be arrested in capillaries by adherence to endothelial cell surfaces and/or the exposed basement membrane. Extravasation and multiplication of tumor cells in the extravascular space of the invaded organs must take place continuously (1). An extremely high rate of tumor cell destruction has been reported in the blood, and only 0 to 1% of injected tumor cells survive and develop macroscopic metastases (2). During the migration in the blood, tumor cells interact with NK cells, activated macrophages, other WBC, complements, and antibodies. Aggregated platelets and fibrin coagulation may also be important in tumor cell arrest in capillaries (3).

The effects of biological response modifier(BRM)-induced NK cell activation in vivo on host resistance against tumor metastases have been reported (4-6). For example, the administration of BRMs, such as Bacillus Calmette-Guerin, Corynebacterium parvum, and poly(I:C)-boosted macrophage and NK cell activities, enhanced the destruction of circulating tumor cells and inhibited lung colonization. The antimetastatic activity was most marked when BRMs were injected before, but not after, intravenous tumor cell inoculation, thus supporting the idea that NK cells and macrophages are most effective in destroying blood-borne tumor cells in the vascular capillary bed before their extravasation into the organ parenchyma (7). However, the direct evidence for the antimetastatic activities of NK cells and activated macrophages has not been presented.

To explore the role of NK cells in tumor growth and metastases, anti-asialo GM1 antibody, a glycolipid surface marker on NK cells, has been employed (8,9). The antibody has been shown to selectively eliminate NK cells, to increase the tumorigenicity of cells in an artificial metastasis model (10,11), and to abrogate

the antimetastatic activity induced by heparin, warfarin, or prostaglandin I_2 (12,13). It has also been claimed that silica exhibits a selective lethal effect on mouse macrophages(14), and that it continuously eliminates NK activity (15,16).

In the preceding section of this thesis, I showed that living and killed intestinal bacteria were effective in reducing colony formation of fibrosarcoma cells in the lung. I also showed that bacterial components, especially lipopolysaccharide (LPS), were effective for this activity. I studied further on the mechanism of the antimetastatic activity of LPS. In this section, I showed that NK cells or macrophages were not involved in the activity, but that the initial arrest of tumor cells was affected by LPS treatment.

MATERIALS AND METHODS

Animals. C3H/HeJ and C3H/HeMsNrs male mice were bred and kept in specific pathogen-free facilities at the National Institute of Radiological Sciences. They were used for experiments at 8 to 12 weeks of age.

Tumors. A weakly immunogenic fibrosarcoma(NFSa), which arose spontaneously in a C3Hf/Kam female mouse, was kept in liquid nitrogen, and the 17th generation of its cells was used for these experiments. Tumors were inoculated into the lower legs of C3H/He mice before use. Single-cell suspensions of tumor cells were prepared as previously described(17). Cells were suspended at an appropriate cell concentration in Hanks' balanced salt solution (HBSS) supplemented with 10 % fetal calf serum (FCS).

Drugs. Rabbit anti-asialo GM1 antibody (Wako Pure Chemical,Osaka) was reconstituted in distilled water, stored at -20°C, and diluted from 1:25 to 1:400 in phosphate buffered saline (PBS) before intravenous injection. Silica (2-10 μ m, BIO-RAD) was suspended in PBS and sonicated before use. Normal rabbit serum (NRS) was diluted 1:25 in PBS before intravenous injection.

LPS of E.cloacae was extracted from dry bacteria with phenol-water as described (18). The LPS preparation contained less than 0.1 % protein, as determined by the Lowry procedure, and no detectable nucleic acid (absorbance at 260nm).

Lung Colony Assays. To produce artificial pulmonary metastases, tumor cells were injected intravenously into mice in an injection volume of 0.5ml of HBSS supplemented with 10% FCS. Mice were killed 11 days after the injection, and the lungs were removed and fixed in Bouin's solution. The number of tumor nodules on the surface of the lungs was counted. The significance of the differences among groups (each group contained 5 mice) was determined using the Wilcoxon test; P value less than 0.05 were

considered significant.

Treatments of Tumor Cells with LPS in Vitro. NFSa cells were plated at a density of $5x10^5$ cells per 75-cm^2 flask, and after cultivation for 2 days, incubated with LPS in MEM supplemented with 10% FCS for a period ranging from 0 to 72 hrs. The cells were washed with MEM and trypsinized for 15 min, to detach them from the flask. After washing 3 times with MEM, the cells were resuspended in MEM supplemented with 10% FCS, and 0.5 ml aliquots of each cell suspension (containing $5x10^4$ cells) were injected into the lateral tail vein of mice.

Staining of Peripheral Blood Leukocytes. Peripheral blood leukocytes were stained immunohistochemically with anti-asialo GM1 antibody. The peripheral blood was obtained by cardiac puncture, and washed twice in PBS. Leukocytes were harvested by centrifugation, suspended in Tris-0.83% NH_4Cl solution, to lyse erythrocytes, washed 3 times in PBS, and smeared on albumin-coated glass slides. The slides were incubated with a 1:50 dilution of anti-asialo GM1 antibody for 30 min. After washing 4 times in PBS for 20 min, the slides were incubated with a 1:40 dilution of peroxidase conjugated goat anti-Rabbit IgG (Jackson Labo.) for a further 30 min. After washing, the color was developed with 3,3'Diaminobenzidine (Wako, Osaka). All incubations were carried out at room temperature. The slides were counterstained with methylgreen(chroma) and mounted with cover slips.

Transfer of Spleen Cells. Spleen cells of mice were suspended in Tris-0.83%NH_4Cl solution to lyse erythrocytes, washed 3 times in HBSS, and suspended in RPMI1640 supplemented with 10 % FCS. The cells ($2x10^7$/ml) were incubated for 1h in the presence of 1 μg/ml LPS and washed 3 times with HBSS. One hundred million($1x10^8$) spleen cells were transferred to recipient mice in a volume of 0.5 ml of RPMI1640 1 day before NFSa challenge ($5x10^4$ cells).

Lung Retention of Tumor Cells. Tumor cells prelabeled in vitro were injected intravenously into mice. The labeling procedures were described previously(19). Briefly, NFSa cells were plated at

$2x10^6$ cells per 75-cm^2 flask in MEM supplemented with 10% FCS. On the following day, the medium was replaced by a fresh one containing 5-[^{125}I]Iodo-2'-deoxyuridine (4 μCi/ml, 1Ci=37GBq; Amersham International Plc.). After 24hr incubation, the cells were treat-ed for 15 min with 0.25% trypsin-0.02% EDTA solution to detach them from the flask, and they were washed 3 times by centrifugation. A suspension containing $5x10^4$ cells in MEM was injected into the tail vein of C3H/He mice. Ten min and 2,4,6, and 24hr after tumor cell inoculation, mice were sacrificed by cervical dislocation, and the lungs were removed to determine radioactivity.

RESULTS

Colony-forming Ability of LPS-treated NFSa Cells in the Lung. To elucidate the mechanism of the antimetastatic activity of LPS, I examined if LPS directly acted on NFSa cells to reduce metastatic activities. NFSa cells were plated and cultured in medium containing either 0.1 or 1.0 μg/ml of LPS, which was effective enough to inhibit metastases. As shown in Table 1, the cells did not change their metastatic ability by the LPS treatment. The result indicates that inhibition of metastases by LPS was not caused by direct interaction between LPS and the tumor cells.

Effects of Anti-asialo GM1 Antibody and Other Treatments on the Development of Pulmonary Metastases. Anti-asialo GM1 antibody, a selective inhibitor for NK cell function in the spleen and blood, was injected intravenously, to learn if NK cells were concerned with the reduction of tumor metastases. As shown in Fig. 1, a dose-dependent increase in the number of lung colonies was observed when the antibody was injected into mice that had not received LPS. However, as shown in Table 2 , anti-asialo GM1 antibody scarcely affected the metastatic rate in LPS-treated to control mice, although it promoted the formation of lung colonies both in control and LPS-treated mice. Similarly, the injection of silica, which inhibits NK cell and macrophage activity only marginally, increased the metastatic rate in LPS-treated mice from 1.4% to 7.5%.

Furthermore, in peripheral blood, the number of asialo GM1-positive cells began to increase as early as day 1 after LPS treatment, and it peaked at day 5, whereas the peak inhibition of metastasis was observed 1-2 days after injection of LPS (Table 3). The results indicate that asialo GM1-positive cells were important for the reduction of tumor cells, but the cells did not concern

the present antimetastatic activity exhibited by LPS.

It was found that the LPS-induced antimetastatic effect was significantly suppressed by whole-body γ-irradiation of 6 Gy 4 days before tumor cell injection, although irradiation with 3 Gy exhibited no significant effect. NK cells and macrophages are known to be resistant to such doses of irradiation. These results suggested that moderate-irradiation-sensitive cells, such as granulocytes, were concerned to the antimetastatic effect exerted by LPS.

Effect of LPS and Anti-asialo GM1 Antibody on The Pulmonary Retention of NFSa Cells. To learn the effect of LPS on the pulmonary retention of tumor cells, NFSa cells were radiolabeled and radioactivity in lung was determined. As shown in Fig. 2, the radioactivity of the cells reduced at a characteristic negative exponential rate, whether or not the mice had received LPS and/or the antibody. Prior administration of LPS enhanced the reduction rate of tumor cells in the lung. At 6 hr after injection, 22.4% of injected cells remained in the lung of control mice, whereas only 11.9% remained in mice treated with LPS. The difference in the rate of retention between control and LPS-treated mice increased at 24 hours; 4% and 0.64% in the control and LPS-treated mice, respectively. On the other hand, anti-asialo GM1 antibody did not affect the retention rate up to 6 hours, and the rate was slightly increased 24 hours after injection of the tumor cells. In the LPS-treated mice, also, anti-asialo GM1 antibody only slightly influenced tumor clearance.

Effect of Transfer of LPS-treated Spleen Cells on Tumor Metastasis. The spleen contains various types of cells that are activated by LPS. Experiments were designed to examine whether LPS-treated spleen cells injected into non treated mice could affect lung metastasis. Spleen cells derived from C3H/He and C3H/HeJ mice were incubated with LPS for 1 hours and injected intravenously to these mice. Significantly lower numbers of tumor colonies were obtained in the lungs of C3H/He mice injected with

LPS-treated spleen cells, regardless of whether the cells were derived from C3H/He or C3H/HeJ mice. In contrast, LPS-treated spleen cells derived from either mice did not influence lung colony formation in LPS low-responder C3H/HeJ mice. The results suggest that LPS, which had been trapped by the spleen cells during incubation, but not spleen cells themselves, was effective in reducting colonies in the lung (Table 4).

DISCUSSION

The mechanism of cancer metastases is a complex cascade of various steps. Interruption of the sequence at any of these steps can prevent the production of metastases. The results of this study, as discussed more precisely later, showed that the antimetastatic effects of LPS were chiefly due to the inhibition of tumor cell retention in the lung, and that NK cells and macrophages, which are thought to be activated by LPS (20-22), were not directly concerned with the present effect of LPS. The LPS effect was eliminated by relatively higher doses of whole-body γ-irradiation, suggesting that the high-irradiation-sensitive cells were concerned in the reduction of tumor migration in the lung.

LPS has been known as a kind of BRM. It activates macrophages and NK cells, and as a result inhibits lung metastases. In fact, in my own experiments, injection of anti-asialo GM1 antibody or silica increased the number of metastases of NFSa cells, and the administration of LPS increased the number of asialo GM1-positive cells in peripheral blood. Treatment with anti-asialo GM1 antibody is known to be one of the most specific methods currently available for the removal of NK cells. The treatment was reported to suppress spleen NK cell activity, to accelerate the initial retention rate of B16 melanoma and MADB106 cells in the lung, and

to increase the number of metastases(10)(12)(13). Treatment of mice with Poly(I:C) and C.parvum augmented spleen NK cell activity and inhibited the initial retention rate of B16 metastases (12).

However, in the present experiment, LPS-induced inhibition of metastases did not coincide with the increase of asialo GM1-positive cells in the peripheral blood. Moreover, neither anti-asialo GM1 antibody nor silica treatments abrogated the antimetastatic activity of LPS. No direct interaction between LPS and NFSa cells was found. Furthermore when LPS-treated spleen cells from C3H/He mice were transferred to C3H/HeJ mice, no antimetastatic effect was induced. These data indicate that activation of NK cells or macrophages had little to do with the LPS-induced inhibition of metastases shown above.

Although most radiolabeled tumor cells were initially arrested in the lung, as shown in the results, they were rapidly eliminated from the lung with or without destruction and only a few cells were sustained in the lung and formed metastatic foci. Retention of tumor cells in the lung is known to coincide well with the incidence of metastases (23,24). However, little is known about the exact mechanisms of the retention or arrest of tumor cells in the lung. Hanna and Fidler reported, using B16 melanoma cells, that the enhanced metastases in mice exhibiting low activity of NK cells is not due to enhanced tumor cell arrest in the lung, but rather to increased tumor cell survival (6). In this study, treatment with anti-asialo GM1 antibody increased the number of metastases, but did not accelerate the initial retention rate of NFSa cells in the lung. Therefore, the reduction of metastasis by NK cells and/or macrophages, and that by LPS, seem to be two independent phenomena. LPS is known to induce various effects other than NK cell activation. These include vascular endothelial damage, pulmonary vascular sequestation of granulocytes, the induction of various lymphokines, etc.(25-29). For the LPS-induced antimetastatic effect in the present experiments, other cell

factors seem of concern, which are inhibitable by whole-body γ -irradiation.

Granulocytes and platelets are known to be concerned with lung injury after the intravenous injection of endotoxin (LPS). These cells are sensitive to whole-body γ-irradiation, in contrast to NK cells and macrophages, which are relatively radio-resistant (33,34). Activated granulocytes possess strong lytic activity against tumor cells (30-32). Platelet aggregation and fibrin formation are considered to be the mechanisms involved in the adhesion of tumor cells to the endothelial surface of blood vessels, tumor cell arrest, and extravasation (28). However, in the present experiments, the injection of LPS 2 hours before tumor cell inoculation did not exhibit antimetastatic effects(See Section I). Acute responses, such as the decrease of granulocytes and platelets, and activation of complements may not be the main factor for the inhibition of metastases (29). A possibility remains, however, that these alterations affected the state or character of endothelial cells or the basement membrane, which in turn affected the arrest of tumor cells in the lung. The mechanism of cancer metastases is compromised and further studies are needed to clarify the mechanism of the antimetastatic effects of LPS.

Table 1 Metastatic capacity of NFSa cells incubated with LPS

After the incubation in LPS-containing medium, NFSa cells were washed 2 times with HBSS, resuspended in HBSS (5x104 cells/0.5ml) and injected intravenously to C3H/He mice. A group of 5 mice was used for each experiment.

LPS	Mean number of lung colonies(range) after incubation for 0h	1h	18h	72h
-	55.4(15-80)	-	-	-
0.1 μg/ml	53.8(34-71)[a]	72.2(51-97)[a]	61.2(47-85)[a]	-
1.0 μg/ml	42.7(30-67)[a]	51.2(24-77)[a]	53.0(27-80)[a]	36.6(21-52)[a]

[a] No significant difference(P>0.05) from control.

Table 2 Antimetastatic effect of LPS in mice treated with anti-asialo GM1 antibody, silica, or whole body irradiation

In experiment I, C3H/He mice received silica(2.5mg/0.4ml PBS) or anti-asialo GM1 antibody (diluted 1:20 with PBS) 1 day before or 1 and 3 days before NFSa cell challenge. LPS(1 μg/0.3ml PBS) or PBS was injected 1 day before tumor challenge. In experiment II, C3H/He mice were irradiated with 3 or 6 Gy γ -rays 4 days before tumor challenge. A group of 5 mice were used for each experiment.

Exp.	Treatment	number of NFSa cells challenged	Mean number of lung colonies(range) in mice received PBS(control)	LPS	%inhibition factor exerted by treatment[b]
I	none	$1x10^5$	56.8(36-70)	0.8(0-4)[a]	-
	Silica	$1x10^4$	106.0(80-122)	8.0(2-14)[a]	1.06
	anti-asGM1(0.2ml/once)	$5x10^4$	192.2(139-224)	7.4(5-11)[a]	1.04
	anti-asGM1(0.4ml/twice)	$2.5x10^4$	161.8(136-177)	0.4(0-1)[a]	1.00
II	none	$1x10^5$	66.2(32-110)	0.4(0-2)[a]	-
	Whole body irradiation(3 Gy)	$1x10^5$	62.2(53-69)	1.0(0-2)[a]	1.01
	Whole body irradiation(6 Gy)	$1x10^5$	132.5(104-154)	50.6(33-75)[a]	1.61

a. $p<0.01$

b. (LPS/control in no treatment group) / (LPS/control in treatment group)

Table 3 Effect of LPS on the induction of asialo GM1 positive cells and on the development of NFSa metastases

LPS (1 μg/0.5 ml in PBS) was injected to C3H/He mice and total WBC and asialo GM1 positive cells in the peripheral blood were counted at the periods indicated. NFSa cells were challenged at the periods indicated and lung colonies were counted 11 days after the challenge.

Day after LPS injection	Mean number of counts(range)[a]		Mean number of lung colonies(range)
	WBC	NO. of asialo GM1 positive cells	
none	4250(3900-4650)	400(380-430)	139.2(63-191)
1	2700(2200-3200)	760(672-870)	1.2(0-2)
3	5300(4300-5800)	2210(2030-2370)	1.2(0-5)
5	7200(7000-7400)	3170(2760-3410)	15.2(6-22)
8	6180(5000-6900)	970(700-1380)	75.0(67-90)
10	4970(4570-5330)	790(750-840)	101.0(73-146)
13	6830(6750-6900)	1080(830-1330)	-

a. Three mice/group.

Table 4 Inhibition of metastases by the adoptive transfer of spleen cells

Spleen cells derived from C3H/He and C3H/HeJ mice ($2x10^7$/ml) were incubated for 1 h at 37°C with or without LPS 1 μg/ml. Washed spleen cells ($1x10^8$) were transferred to recipient mice 1 day before NFSa cell challenge, $5x10^4$ for Exp. I and $1x10^5$ for Exp. II. Each data was obtained from 5 mice.

Experiment	Recipient	Donor	Adoptive transfer	Number of metastases (Range)
I	C3H/He	-	-	69.2(30-107)
	C3H/He	C3H/He	control spleen cells	63.0(40-89)[a]
	C3H/He	C3H/He	LPS-spleen cells	3.8(0-7)[b]
	C3H/HeJ	C3H/He	control spleen cells	117.2(60-165)
	C3H/HeJ	C3H/He	LPS-spleen cells	86.3(57-141)[a]
II	C3H/He	-	-	139.6(121-158)
	C3H/He	C3H/HeJ	control spleen cells	143.4(49-202)[a]
	C3H/He	C3H/HeJ	LPS-spleen cells	33.6(27-39)[b]

[a] Not significant

[b] $P<0.01$

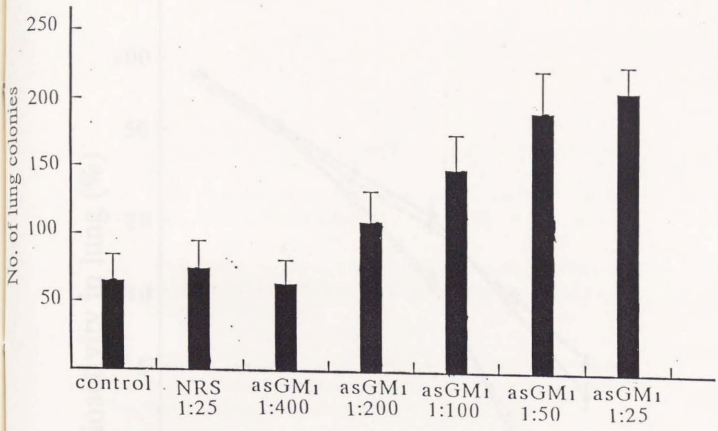

Fig. 1. Effects of anti-asialo GM1 antibody on lung colony formation of NFSa cells. Anti-asialo GM1 antibody(asGM1) in 0.5 ml (indicated dilution) was injected intravenously into C3H/He mice 3 days before $5x10^4$ NFSa challenge. As a control, normal rabbit serum (NRS; 0.5 ml, 1:25 dilution) was injected. Mean value obtained with 5 mice was shown as a column with ±SD as a bar.

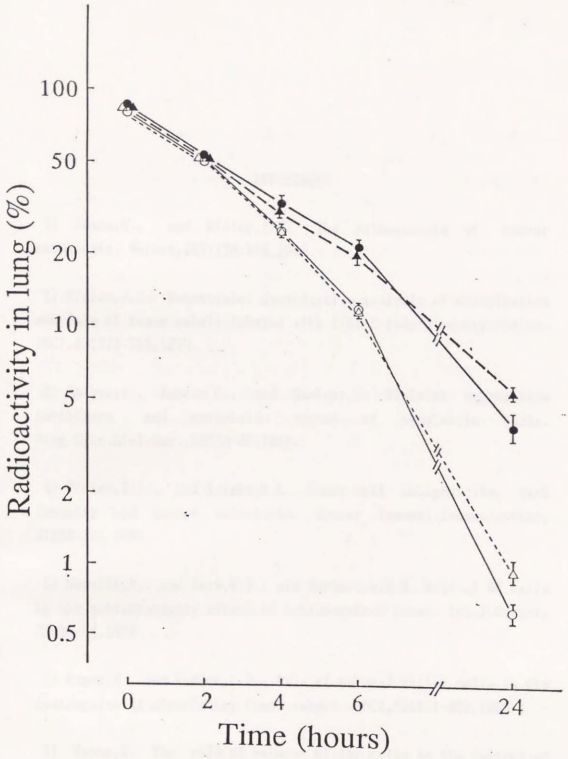

Fig. 2. Effect of LPS and anti-asialo GM1 antibody on pulmonary retention of NFSa cells. C3H/He mice were received LPS(1 μg) or/and anti-asGM1 antibody (0.5 ml, dilution 1:50) or nothing 1 day before intravenous injection of $5x10^4$ radiolabeled NFSa cells. The pulmonary radioactivity was determined 10 min(0 hr), 2,4,6 and 24 hr after tumor cell administration. Results obtained with 4 mice were plotted for each point as symbols and bars(Mean$\pm$SEM). ●,controls; ▲,anti-asialo GM1 antibody; ○,LPS; △,LPS plus anti-asialo GM1 antibody.

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結　論

マウス放射線腹部照射後の肺転移抑制メカニズムについて次の結果を得た。

1．大腸菌を腸内フローラとして持つ動物では放射線照射後、大腸菌が増殖、粘膜を通過、体内へ侵入し転移抑制を引き起こす。

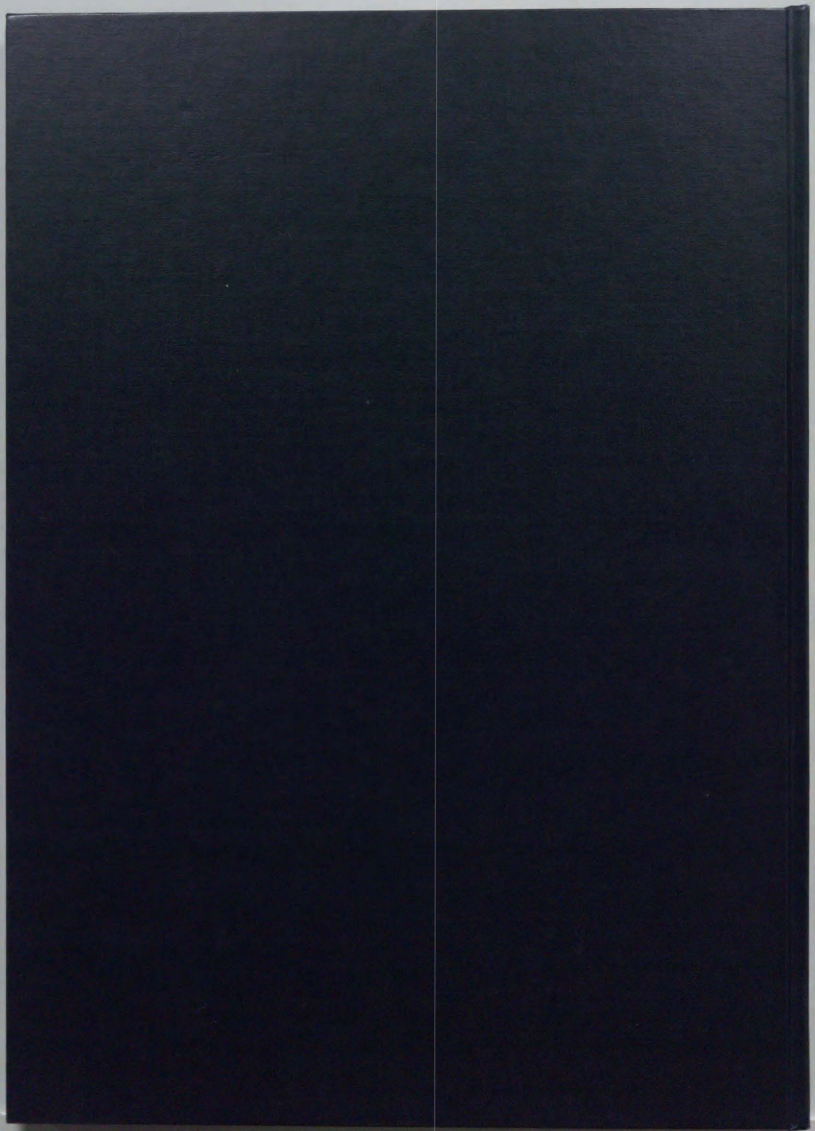
2．肺転移抑制効果は腸内細菌の生菌または死菌をあらかじめ静注しておいても見られ、細胞成分のうち内毒素ＬＰＳが主としてこの効果を発現させる。

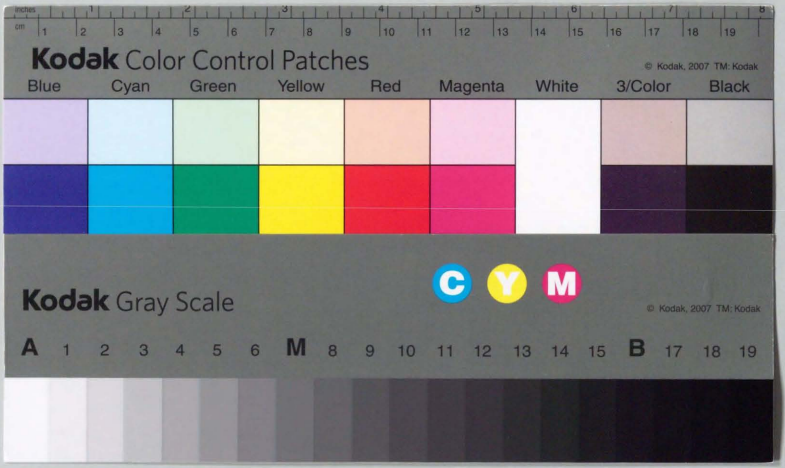
3．ＬＰＳは腫瘍細胞に直接作用せず、マウスに作用して転移抑制を引き起こす。

4．抗アシアロＧＭ１抗体やシリカを静注しＮＫ細胞やマクロファージの活性を抑制すると、肺転移数は著明に増加するが、ＬＰＳによる肺転移抑制率は殆ど影響を受けず、またＬＰＳ投与後マウスの末血中のアシアロＧＭ１陽性細胞と転移抑制能の変動は平衡しないことから、この肺転移抑制にはＮＫ細胞やマクロファージは殆ど関与しないと考えられる。

5．ＬＰＳ静注により腫瘍細胞の毛細血管を通しての定着性が低下するが、抗アシアロＧＭ１抗体は定着性に殆ど影響せず、腫瘍細胞の定着性の変化とＮＫ細胞やマクロファージ抑制による転移の増加はまったく別のメカニズムであると考えられる。

6）全身照射（６Ｇｙ）を受けたマウスではＬＰＳによる転移抑制能が低下し、放射線に感受性のある細胞の関与が示唆される。

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